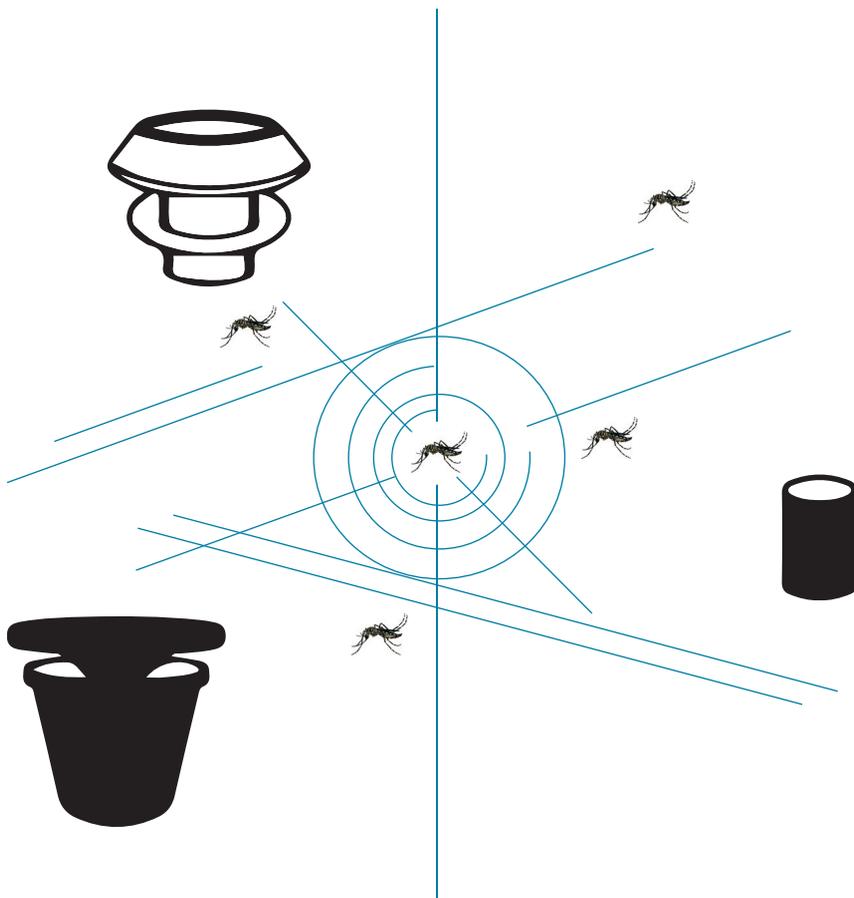# EFFICACY-TESTING OF TRAPS
FOR CONTROL OF *AEDES SPP.*
MOSQUITO VECTORS

World Health Organization

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CONTENTS

ACKNOWLEDGEMENTS

WHO recognizes that as the first guidelines provided for a rapidly developing area of mosquito vector control, we anticipate these to evolve with the field, and actively encourage feedback and suggestions for improvement.

ABBREVIATIONS AND ACRONYMS

ABV	*Aedes*-borne virus
AC50	concentration that attracts 50% of insects
AC90	concentration that attracts 90% of insects
AI	active ingredient
EI	emergence inhibition
EI50	concentration that prevents emergence of 50% of adults
EI90	concentration that prevents emergence of 90% of adults
FT	time to first take-off
IgG ELISA	immunoglobulin G enzyme-linked immunosorbent assay
IGR	insect growth regulator
LC50	concentration that kills 50% of insects
LC90	concentration that kills 90% of insects
NS1	nonstructural protein 1
PCR	polymerase chain reaction
VCAG	WHO Vector Control Advisory Group

GLOSSARY

Active ingredient. The part of a product that has the primary action on the insect (e.g. pesticidal, behavioural, attractant).

Attractant. A biological or chemical (e.g. odorant) or other attractive element (e.g. visual, acoustic) that attracts mosquitos to a trap (also referred to as "bait").

Attractive oviposition trap. Trap designed to attract and kill gravid or ovipositing mosquitos.

Autodissemination. Picking up by adult mosquitos of an active ingredient from treated surfaces of a device or trap and transferring it to aquatic habitats in sufficient quantities to kill larvae or prevent pupae from emerging to adults. Also known as "horizontal transfer (of chemicals)" by mosquitoes (HTM), or "mechanical dissemination by mosquitoes (DSM)".

Autodissemination devices. Devices designed to lure and contaminate mosquitos with a disseminating agent (e.g. an insect growth regulator) for its transfer to additional oviposition sites.

Bait. See "attractant".

Autodisseminant. See "disseminating agent".

Discriminating concentration. Concentration of an insecticide that, during a standard length of exposure, discriminates the proportions of susceptible and resistant phenotypes in a mosquito population.

Disseminating agent (or "autodisseminant"). An active ingredient that is topically picked up by mosquitos from treated surfaces, retained and transferred to aquatic mosquito habitats.

Durability. In relation to vector traps, the physical integrity of a trap and its components over time.

Efficacy. With regards to traps, efficacy is the impact in lowering the mosquito population and/or disease incidence/prevalence in humans.

Efficacy trial. Study to estimate the effect of an intervention under the ideal conditions that can usually be achieved only in a trial, for example, by ensuring maximal coverage of the target population and adherence to the intervention.

Fast-acting insecticide. An insecticide that causes ≥ 80% mortality in susceptible target populations within 24 h of a 30-min exposure to the compound or its active ingredient.

First-in-class. Refers to the first trap for vector control with a novel entomological effect that is validated for public health value by the WHO Vector Control Advisory Group (VCAG) based on demonstration of entomological and epidemiological efficacy.

Incidence. The number of new cases of infection or disease arising in a population per unit time.

Insecticide (see also "Pesticide"). Chemical product (natural or synthetic) that kills insects on contact or by fumigation. Ovicides kill eggs; larvicides kill larvae; pupacides kill pupae; and adulticides kill adult mosquitos. "Residual" insecticides remain active after application. Insecticides can be categorized as fast- or slow-acting.

Insect growth regulator. Compounds such as juvenile hormone analogues (juvenoids) and chitin synthesis inhibitors that prevent the emergence of viable adult insects from larval or pupal stages by disrupting adult development or transformation.

Large-cage studies. Trials conducted in large screened cages or rooms under controlled conditions of temperature and humidity.

Next-in-class (see also "first in class"). Any new subsequent vector trap product having the same mode of action as the first-in-class trap product for which a VCAG recommendation has been made.

Pesticide. Any substance or mixture of chemical or biological agents intended for repelling, destroying or controlling any pest. The term includes microorganisms, insect and plant growth regulators, pesticide synergists and "safeners" that are integral to the satisfactory performance of the pesticide. The term "formulated pesticide" refers to any formulation containing a pesticide (*1*).

Semi-field trials. Trials conducted in screened enclosures in the natural ecosystem of a target disease vector.

Seroincidence. Rate of occurrence of new infections (e.g. number of seroconversions) in the population over a period of time.

Seroprevalence. Proportion of population with serological evidence of a previous infection.

Slow-acting insecticide. An insecticide that has its primary effect on mosquito mortality > 24 h after exposure.

Trap. Structure or device unto which vectors enter and/or make contact with, which ultimately results in their their capture, death and/or sterilization. Traps may work by capturing and retaining mosquitos inside a physical structure ("capture–kill") or by attracting and releasing mosquitos exposed to an insecticide or autodisseminant that will kill, sterilize or otherwise reduce vector populations after individuals leave the trap ("capture–release").

Vector trap for disease control. A trap, as defined above, implemented with the aim of reducing vector density and vectorial capacity and ultimately decreased infection or disease in humans.

Vector trap for surveillance. A trap, as defined above, used to monitor the distribution, abundance and infection rates of vector populations.

1. INTRODUCTION

The geographical distribution of important human disease vectors is expanding, and new vectors and arthropod-borne diseases have emerged. *Aedes aegypti* is the primary vector for many arboviral diseases, including dengue fever, Zika, chikungunya and yellow fever, and is a growing global public health threat. New and improved tools and strategies are needed to suppress vector populations and reduce the transmission of *Aedes*-borne diseases.

Traps are commonly used in vector surveillance to monitor the distribution, abundance and infection rates of vector populations. Several traps have been developed recently with the aim of vector control rather than surveillance; however, there are few trap-based control programmes, and evidence of a demonstrable effect in the field is required. Traps could help to reduce disease transmission by lowering vector densities below a transmission threshold or selectively targeting the older female mosquitos responsible for transmission, shifting the age structure and reducing the abundance of infectious vectors.

The purpose of this document is to provide procedures and criteria for testing the efficacy of and evaluating vector traps for disease control. It includes the design of laboratory and small-scale field trials to assess the attraction and killing effects of vector traps and of large-scale community trials to determine the efficacy of traps in reducing mosquito populations in the field and disease transmission. This document focuses on traps for container-inhabiting *Aedes* spp. mosquitos (*Ae. aegypti* and *Ae. albopictus*). Other species of mosquitos, with different larval aquatic habitats (e.g. *Anopheles*, *Culex*, floodwater mosquitos), are not yet included; however, the general testing framework described could be extended to other traps after some modification, including those for other vector species.

Vector traps are devices into which vectors enter or otherwise make contact, which ultimately result in their death or sterilization. Traps target different stages of mosquito life (eggs, larvae, pupae or adults) or physiological stages (e.g. host-seeking or gravid females). The ability of traps to attract vectors may be a function of their physical design or chemical attractant; similarly, killing may be achieved through physical design with or without insecticides. In this document, the strategy of killing vectors in traps is referred to as "capture–kill", whereby mosquitos that enter the trap are physically confined and exposed to a "fast-acting" chemical or biological insecticide (illustrated in **Fig. 1**). The trapping strategy whereby mosquitos enter the trap, come in contact with an insecticidal or sterilizing agent and then leave the trap are referred to as "capture–release" (*2*). In an autodissemination strategy, adult capture and exposure are amplified by transfer of the disseminating agent to wider aquatic habitats, where it kills larvae or prevent adults from emerging (*3*).

The WHO Vector Control Advisory Group (VCAG) has reviewed initial evidence on two broad classes of traps for control of *Aedes* vector populations: adulticidal oviposition traps, which target gravid female mosquitos (*4, 5*), and autodissemination devices, in which gravid adult females attracted to traps are contaminated with a "slow-kill" insecticide and a larvicide (IGR) for dissemination (*6*). Other traps, with new designs, attractants and insecticides, are being developed by manufacturers. The efficacy claims of new traps (on

Fig. 1. Vector traps covered in this document
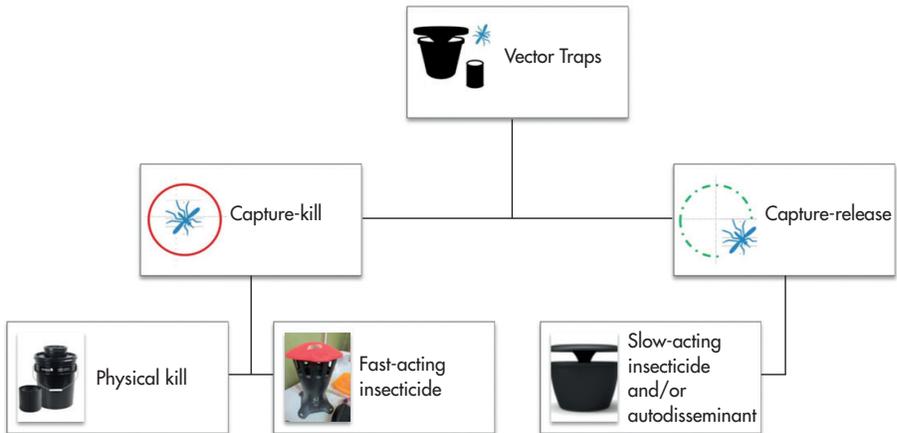
the product label or elsewhere) must be validated, and the traps shown to adequately reduce *Aedes* populations and *Aedes*-borne disease before WHO can issue a policy recommendation for the broad public health use of traps for vector control. Once a policy recommendation has been developed, it is envisioned that vector trap products that are "next-in-class", thus having the same mode of action as a first-in-class product, will be assessed on entomological data only, and in most cases will not need to present epidemiological data for assessment.

This document, prepared in response to recommendations of VCAG, is intended to provide support to product developers, programmes and testing institutions in generating robust entomological evidence of the efficacy of vector traps for control and, for a first-in-class vector trap, evidence of the public health impact in reducing arboviral disease. The guidelines will be the basis for WHO evaluation of new traps and assist countries in testing the effectiveness of traps for vector control locally. The guidelines may be modified once proof of principle is established (i.e. the public health value of vector traps for controlling vector-borne disease) and as new designs, attractants, insecticides and test methods become available.

With the rapid spread of *Aedes*-borne arboviral diseases, new tools for selective targeting and suppression of *Aedes* populations are required to protect people living in areas of risk. Traps and target-based strategies have been used successfully to reduce tsetse-borne trypanosomiasis (*7*). If vector traps are proven to be effective, they could supplement current methods and improve control of *Aedes*-borne arboviral diseases. Vector traps will be most effective when used as one component in a package of interventions, and when implemented by control programmes to ensure proper use, monitoring, servicing and deployment coverage to have the desired effects on mosquitos and disease.

2. GENERAL CONSIDERATIONS FOR TESTING

As biological tests are subject to variation, they should be conducted under the close supervision of personnel who are familiar with methods for testing vector control products and compounds, using sound scientific and experimental procedures. Use of standard operating procedures for testing and for data processing, management and validation is advisable, and training of laboratory and field personnel should be documented. WHO recommends testing according to good laboratory practice as defined by the Organisation for Economic Co-operation and Development (2). When possible, testing institutions certified as adhering to good laboratory practice should be used for testing vector traps for WHO evaluation and prequalification listing.[1]

These guidelines are designed for evaluation of whole traps and associated attractants and/or insecticides that have already been assessed for risk and hazard. It is important that, before testing, investigators review material safety data sheets, draft product labels and certificates of compliance with manufacturing specifications and any supporting data. Independent physical and chemical assessment for compliance with the manufacturer's product specifications may be required.

Data should be collected and reported in such a way as to allow comparisons among numerous evaluation sites. For field trials, the number of replicates should be based on sample size estimates to ensure that a statistical evaluation has enough power to demonstrate efficacy. At a minimum, the data to be reported are a measure of centrality (e.g. mean, median or proportion), sample size and a measure of variability (e.g. standard error, 95% confidence interval or interquartile range).

Evaluations of vector traps should be conducted in accordance with applicable national ethical regulations, including experimental use permits for field trials. Any adverse effects on humans or potential non-target effects during relevant phases of testing should be recorded and reported.

The criteria and methods described in these guidelines will be updated by WHO as new traps, assessment methods and efficacy data become available. The test requirements for vector traps are summarized in **Table 1**.

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1. The WHO prequalification team for vector control products, also known as PQT-VC, should be consulted for advice on risk assessments, specifications and prequalification requirements (http://www.who.int/pq-vector-control/en/, accessed September 2018).

Table 1. Types of studies for testing the efficacy of vector traps

Testing stage	Outcome or end-point	Applicable to	Indicator
Laboratory studies	Intrinsic activities of new AIs	New AIs only	LC_{50} and LC_{90} (both adulticides and larvicides), IE_{50} and IE_{90} (IGR), AC_{50} and AC_{90} (attractants)
	Excito-repellency	New AIs only	FT_{50} and FT_{90}
	Transfer of autodisseminant	New AI for auto-dissemination only	LC_{50} or LC_{90} or EI of susceptible larvae exposed via transfer of the autodisseminant
	Discriminating concentration	New AIs only	Discriminating concentration of AI
	Cross-resistance	New AIs only	Cross-resistance to other insecticides in unrelated insecticide classes
	Bioefficacy of formulation	All traps, AI formulations	Percentage efficacy and duration efficacy is maintained to product claims
Contained and small-scale field trials	Trap efficacy	All traps: CK, CR	Immediate and delayed mortality (adults and/or larvae) or EI
		All traps: CK, CR	Trap oviposition rates (# eggs per trap)
		CR traps only	Adult EI (%) from secondary containers[a]
	Effective trap duration	All traps: CK, CR	Number of days or weeks during which efficacy end-points meet product claims
	Effective trap density	All traps: CK, CR	Optimal number of traps per unit area
Field trials for entomological end-points	Entomological efficacy in the field	All traps: CK, CR	Significant difference in mosquito population density between treated and control areas
		All traps: CK, CR	Significant decrease in proportion of older female (parous) mosquitos
	Durability and attrition	All traps: CK, CR	Day on which efficacy indicators are not different from no trap
	Non-target effects	All traps: CK, CR	Observed negative effects on non-target organisms
Community trials for epidemiological end-points	Public health efficacy	First-in-class only	Target disease incidence or transmission Entomological outcomes (above) Community perceptions and acceptance of the intervention Adverse events per person exposed to traps and/or control

AC, attractant concentration; AI, active ingredient; CK, capture–kill; CR, capture–release; EI, emergence inhibition; FT, time to first take-off; LC, lethal concentration;

[a] This step is required only when traps include an autodissemination component.

3. LABORATORY STUDIES

Laboratory studies include tests on new active ingredients (AIs) and formulated products only. The efficacy of whole traps is studied in contained and small-scale field trials (section 4) and in large-scale field testing (section 5).

For vector traps, laboratory studies determine the intrinsic biological activity of new active ingredient(s) used in the traps, discriminating concentration and any cross-resistance with known insecticide resistance mechanisms. Laboratory studies also include determination of the efficacy and residual activity of formulated trap component products

The following objectives are relevant only for new molecules (AIs) for which evidence in the target vector has not been previously generated:

- to establish dose–response relations and determine the lethal concentration (LC) of fast- and slow-acting insecticides for 50% (LC_{50}) and 90% (LC_{90}) mortality or emergence inhibition (EI) of susceptible larval and adult mosquitos;
- to establish dose–response relations and determine the attractant concentration (AC) of a bait active for 50% (AC_{50}) and 90% (AC_{90}) attraction of mosquitos towards a chemical stimulus;
- to determine the "time to first take-off" (FT) for 50% (FT_{50}) and 90% (FT_{90}) of mosquitos after exposure to the insecticide-treated substrate;
- to establish the dose–response relation of an AI for autodissemination on adult mosquitos to achieve LC_{50} and LC_{90} of susceptible mosquito larvae that are exposed by transfer of the autodisseminant from the adult to the larval habitat;
- to assess cross-resistance of the insecticide against unrelated classes of insecticide; and
- to establish discriminating concentrations for monitoring susceptibility.[1]

Additionally, for formulated trap component products, the objective is to determine the efficacy and residual activity of a formulated AI or other agent (e.g. adulticide-treated netting, larvicide product).

3.1 GENERAL CONSIDERATIONS FOR TESTING

In order to standardize test outcomes at the laboratory stage as far as possible, laboratory tests should be conducted on well-characterized susceptible laboratory strains of *Ae. aegypti* or *Ae. albopictus*. The mosquito species and colony strain used in the test must be reported. If tests are done with other species of vectors (e.g. *Anopheles* or *Culex*), well-characterized laboratory strains should also be used and the species and colony strain reported.

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1. Discriminating concentrations are already known for many insecticides. They should be determined only when they are not yet known for the target vector species.

Standardized mosquito rearing and testing conditions are essential to ensure the reliability and reproducibility of data. Existing institutional standard operating procedures (8–11) should be followed or adapted as necessary. Mosquitos are usually reared at 27 °C ± 2 °C, at 80% ± 10% relative humidity and a 12:12 h light:dark photoperiod. Test mosquitos are maintained on sugar meals (e.g. 10% sucrose) and can be non-blood-fed or blood-fed, depending on the mosquito physiological stage that is targeted by the trap. Most ovitrap AIs and components should be evaluated in 6–8-day-old gravid female mosquitos that took their first blood meal 2–4 days before the experiments. Host-seeking mosquitos are usually 3–5 day-old non-blood-fed females that have been sugar-starved for 24 h. Institutional protocols should be followed for rearing mosquitos to the desired physiological stage.

When possible, each test should include a negative control, with no insecticide or attractant, and a positive control, such as a reference attractant or insecticide for which there are data.

Equipment must be thoroughly cleaned between tests to ensure that residual material does not bias the test results.

3.2   ASSESSMENT OF INTRINSIC ACTIVITY

Intrinsic activities are assessed for novel AIs only when the biological activity against mosquitoes has not already been shown. The tests are not relevant for non-chemical components that are not produced to a manufacturing standard (e.g. hay infusion as attractant) or for formulated products (e.g. treated netting, water-soluble larvicide granules) used in traps. The relevant testing methods are summarized below.[1]

3.2.1 ADULTICIDES

To evaluate the intrinsic biological activity of a mosquito adulticide, laboratory-reared adult female mosquitos are exposed to a range of concentrations of the AI applied topically, and mortality is recorded. Topical application is used to differentiate the toxicity from confounding effects on insect behaviour. Details of testing procedures for intrinsic activity can be found in the WHO guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets (*12*); refer to section 2.1 of referenced guidelines and relevant SOPs.   The bioassay procedures are the same for slow-acting as for fast-acting adulticides, except that, for the latter, mortality is monitored every 24 h until the full effect has been achieved.

3.2.2 LARVICIDES

The objective is to measure the inherent biopotency of a mosquito larvicide against the target species. Laboratory-reared mosquito larvae are exposed to a range of

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1. For each referenced WHO guidelines, the most recent version should be followed and, where available, the relevant WHO standard operating procedures.

concentrations, and mortality or EI is recorded. Details of testing procedures and larval bioassays can be found in the WHO guidelines for testing mosquito larvicides (*13*); refer to section 2.1 of referenced guidelines and relevant SOPs.

3.2.3 ATTRACTANTS

The intrinsic activity of attractant AIs (including dose-response) may be a critical component of trap efficacy. Evidence should be provided demonstrating the basic ability of a new synthetic active ingredient to attract mosquitos. Laboratory-reared adult mosquitos are exposed to at least five concentrations within the activity range defined by the manufacturer or published literature. Attraction is measured in a Y-tube olfactometer in the absence and presence of the candidate compound. Tests are done on host-seeking or gravid mosquitos, depending on the physiological state targeted. Details on testing procedures can be found in WHO guidelines for testing spatial repellents (*25*); refer to section 2.1 of referenced guidelines and any relevant SOPs. These methods may need further development and validation as new attractant molecules are brought forward for use in vector control.

3.2.4 ASSESSMENT OF EXCITO-REPELLENT ACTIVITY

The repellent and irritant effects of an insecticide can modify the tarsal contact time with a treated substrate, which may reduce the lethal effect of an adulticide or reduce the probability that adult mosquitos will be contaminated and subsequently transfer the autodissemination agent. WHO cone assays may be used to assess the time between first landing and take-off for individual mosquitos exposed to technical-grade insecticide on filter paper and relevant formulations. Further details and test procedures are described in reference (12); refer to section 2.3 of referenced guidelines and relevant SOPs.

3.2.5 AUTODISSEMINATION

For AIs and formulations for autodissemination, modified bottle bioassays can provide information on the transfer of an AI to the adult mosquito (see supplemental materials). In brief, adult females are exposed to concentrations of the autodisseminant in glass bottles and then placed in screened cages with bioassay containers holding susceptible immature mosquitos. Larval mortality or EI is measured to establish the dose–response relation for 50% and 90% mortality in susceptible larvae.

Additional development and validation of bioassays may be required for different autodissemination agents and to evaluate other effects on mosquito physiology, such as chemosterilization. Suggested efficacy indicators for chemosterilization include total and mean number of eggs laid, hatchability and oviposition inhibition.[1]

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1. The 2018 WHO guidelines for laboratory and field-testing of long-lasting insecticidal nets currently under development and related SOPs should be consulted for details on assessment of reproductive output.

3.3 DISCRIMINATING CONCENTRATION AND CROSS-RESISTANCE

Discriminating concentrations of all new insecticides for vector control are required for monitoring insecticide resistance in the vectors and to assess whether an intervention will be effective against local mosquitos (*14*). Test procedures should accord with WHO standard procedures for establishing discriminating concentrations (*15*).

New AIs submitted for evaluation should also be tested to determine whether there is cross-resistance with known resistance mechanisms. New compounds can first be tested against multiresistant strains of mosquitos and then against insect strains carrying one or more resistance mechanisms, as per WHO guidance (*12, 15*).

3.4 BIOEFFICACY AND RESIDUAL ACTIVITY OF FORMULATED PRODUCTS

Before testing a whole trap in small-scale field studies, the efficacy of formulated products in traps (e.g. adulticide-treated netting, sticky surface inserts, attractant sachets, larvicides or other trap components) should be validated in controlled laboratory studies. Tests should measure the initial efficacy against laboratory-reared, susceptible mosquito populations of the targeted physiological stage (e.g. gravid females, host-seeking females, larvae) and verify the proposed duration of efficacy of the products. For adulticide-treated trap components, methods can be adapted from cone bioassays (*12*, section 2.4.2). Formulated larvicides and insect growth regulators should be in accordance with laboratory methods described in the WHO guidelines for testing mosquito larvicides (*13*).

Procedures for testing attractant formulations, autodisseminants and sticky surfaces may require additional development and validation of published bioassays (e.g. *16–19*).

3.5 STATISTICAL METHODS AND DATA ANALYSIS

An appropriate estimate of centrality (mean, 95% confidence interval or median, interquartile range) are calculated and reported for the outcomes. The activity of the test compound (e.g. adulticide, larvicide) against a particular vector strain can then be compared with values for other compounds.

3.5.1 ADULTICIDES, LARVICIDES, INSECTICIDE GROWTH REGULATORS, ATTRACTANTS

The relation between dose and mortality can be analysed by log–dose probit regression with relevant statistical software packages to estimate LC_{50}, LC_{90} (or AC_{50}, AC_{90}) and 95% confidence intervals.

For insect growth regulators, total or mean emergence inhibition (EI) can be calculated from the number of larvae exposed and the overall emergence of adults. EI is calculated from:

$$EI\,(\%) = 100 - \frac{T \times 100}{(1/C)}$$

where T = percentage survival or emergence in treated batches and C = percentage survival or emergence in the control.

3.5.2 REPELLENT AND EXCITO-REPELLENT ACTIVITY

The relation between dose and percentage repellent and take-off due to irritability (excito-repellency) is analysed by log–dose probit regression.

3.5.3 DIAGNOSTIC CONCENTRATION

The diagnostic or discriminating concentration is determined from the dose–response regression lines obtained by testing a technical material in a susceptible vector species. The diagnostic concentration is double that of the estimated $LC_{99.9}$ estimated by probit.

3.5.4 CROSS-RESISTANCE

The LC_{50} value for susceptible mosquito strain is compared with those for several resistant strains to estimate the existence and level of cross-resistance (resistance ratio of 50% or 95%) of the new candidate insecticide (*20*).

3.6   INDICATORS FOR LABORATORY STUDIES

The values listed below should be reported where appropriate from laboratory tests.

For new AI molecules for use in vector traps:

- intrinsic activity: LC_{50} and LC_{90} (both adulticides and larvicides); EI_{50} and EI_{90} (insect growth regulators); AC_{50} and AC_{90} (attractants);
- excito-repellency: FT_{50} and FT_{90};
- transfer of autodisseminant: LC_{50} or LC_{90} or EI_{50} or EI_{90} of larvae exposed via transfer of the autodisseminant;
- discriminating concentration of AI; and
- cross-resistance to insecticides in unrelated classes.

For all formulated components for vector traps:

- bioefficacy of formulation: % mortality, EI or attraction of the target mosquito in the laboratory and the number of days the effect is maintained, according to product claims.

4. SMALL-SCALE FIELD TESTING (CONTAINED AND OPEN-FIELD TRIALS)

Small-scale, controlled evaluations of whole traps are performed with target mosquitos under contained field conditions and in small open field studies. Data collected in this phase are used to validate the claims of the manufacturer regarding efficacy and use, and to plan the next phase of testing in large-scale efficacy trials. The aims of small-scale studies are to determine the efficacy and duration of the effect of the whole trap against target vectors under controlled conditions and the effective trap application density (i.e. number of traps per unit area).

4.1 GENERAL CONSIDERATIONS FOR TESTING

4.1.1 TEST SET-UP FOR SMALL-SCALE TRIALS

Traps can be tested in large-cage or semi-field systems (contained trials) to simulate indoor or outdoor use conditions or in small-scale open field trials, depending on the end-point. Contained trials have the advantage of involving laboratory-reared mosquitos (reference or F1 of field-collected mosquitos) that are pathogen-free and of known age and physiological condition (e.g. gravid). Tests of oviposition traps in large cage and semi-field system experiments have shown good correlation with data from field tests; however, some end-points, such as effective trap density, can be measured only in open-field studies (**Table 2**). To estimate the duration of trap activity, traps are exposed to conditions of natural use (e.g. temperature, sunlight) and retested in contained trials or small-scale open field trials at set times after first use to measure their efficacy over time.

- **Large-cage trials in laboratory enclosures**: trials conducted in screened enclosures or free-flight rooms in a controlled laboratory environment with set temperature, light, humidity and air movement (*21–23*).
- **Semi-field trials in natural ecosystems**: trials conducted in screened enclosures in the natural ecosystem of the target disease vector, in local conditions of ambient temperature, light, humidity and air movement. The environment should emulate the natural habitats of the target vectors (e.g. with endemic plants and vegetation, artificial containers) (*24–25*).
- **Small-scale open-field trials**: trials conducted in local settings at limited scale, e.g. a single village. They allow collection of data on end-points that may not be feasible in enclosed studies, such as effective trap density. Small-scale field trials should be conducted in settings that represent the environments in which traps are to be deployed (e.g. back yards, in and around houses) and where the target vector is endemic.

Table 2. Small-scale contained and open-field studies on vector traps

End-point	Evaluation	Trap type	Indicator
Trap efficacy	Large cage, semi-field	Capture–kill / capture–release	Immediate and delayed mortality (adults and/or larvae) or EI
		Capture–kill / capture–release	Trap oviposition rates (number of eggs per trap)
		Capture–release	Adult EI from secondary containers (dissemination)
Effective trap duration	Large cage, semi-field and open field	Capture–kill / capture–release	Number of days or weeks for which efficacy end-points meet product claims
Effective trap density	Open field	Capture–kill / capture–release	Optimal number of traps per unit area

EI, emergence inhibition

4.1.2 MOSQUITOS AND COLLECTION METHODS

For trials in large cages or semi-field systems, well-characterized laboratory-reared strains or F1 generation offspring of mosquitoes collected in the field should be used. Appropriate arthropod containment guidelines should be followed (*26*). For open-field trials, traps are assessed against local field populations of mosquitos at trial sites.

For contained trials, it is important to be consistent in the timing of mosquito release and data collection. Ideally, trials are conducted in the afternoon, with mosquitos released around 16:00 h and the traps monitored the following morning to minimize heat stress on the mosquitos. Running contained trials for longer should be justified in the trial protocol. For traps targeting gravid mosquitos, 6–8-day-old gravid females that took their first blood meal 2–4 days before the experiments and held with access to a sugar solution can be used. Tests of traps for host-seeking mosquitos can involve 3–5-day-old nulliparous females that have been starved of sugar solution for 24 h. The conditions under which mosquitos are reared and held before use in experiments should be recorded, as this may influence the efficacy of traps (supplemental materials).

Trials in large cages or semi-field systems should aim to recapture recapture all mosquitos that were released so that the investigators can calculate the percentage mortality (including delayed mortality) and remove the remaining mosquitos via aspiration before further bioassays. Large cages or semi-field systems should be designed to allow collection of released mosquitos, through use of white netting, lowered ceilings, careful sealing of release chambers or placement of refuges such as black cloth-lined resting boxes in semi-field systems. It may also be necessary to use ant channels and daily cleaning to prevent scavenging of dead mosquitos. In all trials of this nature, there should be a wash-out period or other means of clearing all mosquitos between trials if they are not recovered through aspiration.

Trained technicians skilled in the use of aspirators should perform collections, aiming to catch all mosquitos, knocked down or resting. The total number of recaptured mosquitos should be recorded to indicate if there is some unaccounted loss. Resting mosquitos can be captured with mechanical aspirators, sweep-nets or other methods and sampling repeated until, as far as possible, all the released mosquitos are recaptured.

4.1.3 STUDY DESIGN CONSIDERATIONS

For longitudinal trap evaluations, it is important to sample systematically (e.g. weekly) throughout the test period. It is advisable to monitor the fitness (response to odour cues, egg laying or retention) of the released mosquitos. Wind speed and direction, temperature, relative humidity and precipitation should be recorded for each trial. Care should be taken to mount instruments out of direct sunlight, in the same location in each compartment for consistent comparisons of measurements. Between evaluations, products should be stored according to the label instructions or under environmental conditions similar to those used for evaluating the traps.

Experimental controls should be considered carefully. A standard negative control should be used in planned efficacy trials; for example, for gravid mosquitos, a black 1-L container with 400 mL of deionized water is suggested. Alternatively, permutations of the trap with and without AIs can be used. Currently, there is no standardized active comparator for traps; however, commonly used surveillance traps for which published efficacy data are available, could be used as a reference to compare the performance of other traps.

The number of replicates per product evaluated should be based on sample size estimates, which are required to ensure that a statistical evaluation has sufficient power (*27*). It is highly desirable that the study be fully randomized and that all field operatives be "blinded" to the allocation of treatments in order to avoid bias in the evaluation. If blinding is not possible because of the characteristics of the product (e.g. odour, colour), data should be blinded before analysis (*28*).

4.2 EVALUATION OF TRAP EFFICACY AND EFFECTIVE TRAP DURATION

Trap efficacy is assessed for candidate traps in semi-field settings or large-cage or free-flight rooms. The primary efficacy indicators are adult and/or larval mortality or EI, and trap oviposition. Where relevant, dissemination efficacy is indicated by mortality or EI from secondary oviposition containers.

To measure adult and/or larval mortality (or EI), the candidate trap is tested against a control trap in a no-choice test (i.e. either the candidate test trap or the negative control is used in one of two experimental areas or chambers). Choice tests, in which mosquitos choose between a test trap and a control in the same experimental area, are used when measuring oviposition in traps and autodissemination efficacy.

Factors that could influence preference for a trap, such as location bias, should be controlled (e.g. in a Latin square design where possible (*29*)). Care should be taken to place the traps in the same way and out of direct sunlight so as not to alter their attractiveness or the efficacy of the AIs. If multiple traps are used in choice assays, trap distance – especially with attractants – should be considered, to account for interference among traps. Gravid test traps can be placed equidistantly at a minimum of 1 m apart, while host-seeking mosquito traps can be separated by longer distances (e.g. 10 m), depending on the product claims (*30*). Mosquitos can be released in the centre of the set-up so that they have an equal probability of encountering any of the traps (test or control). Traps can be labelled with unique identification numbers and assigned randomly to an experiment or sampling station with a random number generator.

The number of replicates should be determined a priori by sample size calculation. For each replicate, at least 50 mosquitos (reared as described in section 4.1.1) are released into each large cage or semi-field compartment. For autodissemination trap trials, a maximum of 50 mosquitos should be used for each replicate. Each trial is terminated after the exposure time (usually the following morning or after 24 h). Standardized start and end times for trap operation should be used and recorded on data forms (see example in supplemental materials). At the end of the contained trial, the investigators should recapture all mosquitos, both in and outside traps, and record their status (alive, dead, gravid). A minimum recapture of 50% of released females is required for an assay to be valid.

4.2.1 MORTALITY – ADULTS IN TRAPS

For capture–kill traps, such as sticky traps or traps that prevent mosquitos from exiting, adults retained in the traps should be identified and counted. For traps in which mosquitos are killed with an adulticide, recaptured mosquitos (in and outside the trap) may be held under optimum conditions, i.e. 27 ± 2 °C and 80 ± 20% relative humidity, for a standard period defined by the AI to measure mortality after the specified holding period, e.g. 24–72 h. The performance of the trap, as measured by the proportion of retained mosquitos (percentage of females trapped) or mortality (percentage of females dead per trap), is compared with that of a negative control, in which mortality should not exceed 20%. Traps designed to kill mosquitos by retention should be monitored for mosquito escape by appropriate methods, such as video recording or holding traps in small cages.

4.2.2 MORTALITY – LARVAE IN TRAPS

In traps intended to kill larval stages and prevent adult emergence, females are allowed to lay eggs, and the performance of the trap is measured by the number of eggs laid and the percentage hatching, larval mortality and/or EI. The maximum acceptable mortality in the control is 20%, and emergence in the control group should be 80% for the test to be valid.

4.2.3 ATTRACTION – OVIPOSITION

To measure attraction to ovipositing females and to rule out repellency, the performance of a trap can be measured in an oviposition choice test, in which an alternative oviposition container is provided, such as black, 1-L pots each holding a clear glass bowl with 400 mL of water (6). The number of eggs laid in each oviposition site (candidate trap and secondary container) is used to calculate the percentage of eggs in the candidate trap and in the water-only controls.

If standardized recording of the first choice of oviposition location for *Ae. aegypti* is required, an additive (e.g. 0.07% Aquatain silicone oil) is applied to lower the surface tension of the water, which will cause female mosquitos to drown while ovipositing; however, the compound should be carefully selected to ensure that it does not deter ovipositing females. The performance of the trap is compared with that of the negative control and, if relevant, a standard (positive control).

4.2.4 AUTODISSEMINATION

The efficacy of autodissemination traps and devices for killing mosquitos can be measured as described above. To avoid contamination, treatment and control traps should be tested in separate testing compartments (e.g. semi-field, large cage, free-flight room).

The efficacy in disseminating insecticide to secondary (or alternative) oviposition sites is measured in a choice test, in which two alternative oviposition sites (also called "secondary containers") are provided. Secondary containers, such as black, 1-L pots each holding a clear glass bowl with 400 mL of water (6), can be placed at fixed locations a minimum of 1 m from the dissemination device, with two secondary containers per device.

To assess the efficacy of dissemination, 25 *Ae. aegypti* larvae (late L3 or early L4) and a larval food source are added to each secondary container. The following morning (or after a specified interval such as 24 h), the containers with larvae are removed and larval mortality and EI are monitored in the laboratory (13). The presence of eggs in all available oviposition sites is recorded.

Care should be taken when setting up an experiment to avoid contamination of secondary containers or control traps by handling, for instance by changing gloves between handling devices and decontamination procedures for moving devices between experimental compartments.

4.2.4 DURATION OF TRAP ACTIVITY

In order to evaluate the duration of efficacy, traps should be tested (mortality, capture, oviposition, dissemination efficiency) weekly to determine whether the efficacy targets are met, either for the duration specified on the product label or, if no claim has been made, the day on which the efficacy target falls below 50% of the initial level. Between tests, traps should be stored under normal conditions of temperature and sunlight.

4.3 EVALUATION OF TRAP DENSITY

The likelihood that an individual mosquito will come into contact with a vector trap depends on the local abundance of mosquitos, their habitat, the presence of competing aquatic sites and also the number of traps deployed in a given area. Trap density is an important consideration for efficient deployment, as a high density of traps might be expected to maximize the likelihood of mosquito capture, but reducing trap density would lower costs. The number of traps required in an area depends on the type of larval habitat, density of houses, housing characteristics, mosquito species and amount of open space available. Small-scale open-field trials are conducted to confirm the proposed trap density (number of traps per unit area) before large-scale field testing of traps.

The number of traps per defined area (a back-yard, for example) can be studied by comparing the trap capture rate with increasing trap densities (one, two, three or four traps per area). The optimal number of traps is reached when the number of mosquitos captured per trap reaches a plateau (**Fig. 2**). Trap density should be evaluated in both rainy and dry seasons, especially for devices that mimic oviposition sites, as they compete with larval development sites.

For autodissemination devices, the number of devices needed in a defined area can be

Fig. 2. Measurement of trap density: (A) boxes represent defined areas with an increasing number of traps (circles); (B) in this example, the number of traps that capture mosquitos in each area plateaus at three traps (dashed vertical line), indicating the optimal number of traps to be used. This graph represents a predicted relationship assuming constant recruitment into mosquito population and density of competing attractants (e.g. host sources); the relationship between trap density and catch would also be influenced by these factors.
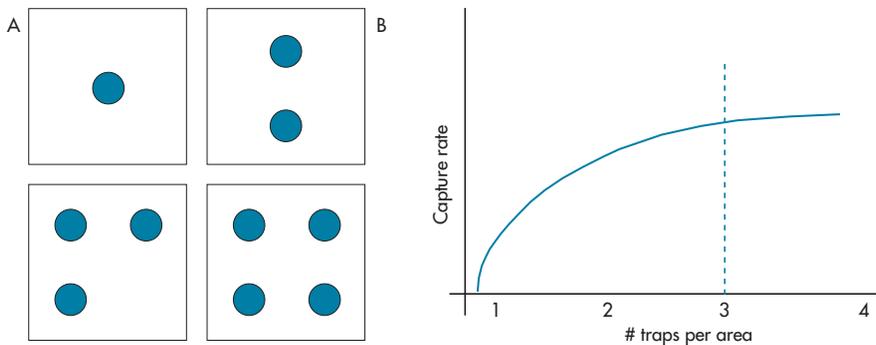
estimated from the larval and pupal mortality and EI in sentinel aquatic sites (secondary containers) placed at known distances from the candidate autodissemination device, compared with similarly set-up in uncontaminated control test sites. As in contained testing assays (section 4.2.4), larval bowls from sentinel sites are taken back to the laboratory for bioassay.

4.4 STATISTICAL ANALYSIS

The primary analysis should be a comparison of a candidate trap with a negative control. The statistical approach should include control for clustering and sources of variation in the experiment, such as replicate or location, in a mixed-effects or generalized linear model (i.e. distribution of families that are not necessarily normal).

Measures of centrality (e.g. mean, median, proportion) should be presented, with 95% confidence intervals or interquartile range, in addition to the results of statistical analysis, by giving the coefficient or odds ratio, value of the test statistic, associated P value and degrees of freedom.

Many of the outcome variables measured in laboratory experiments are proportions (e.g. proportion of adults or larvae dead). These data can be analysed in a binomial model, but the denominator must be specified. Other variables measured are counts (e.g. number of eggs laid, number of captured mosquitos), which can be modelled with a Poisson or negative binomial distribution, depending on the degree of overdispersion. For slow-kill AIs, daily mortality rates can be assessed by Kaplan-Meier or Cox regression to determine whether the survival of the test groups differs significantly.

Other appropriate tests include probit analysis to calculate the LC_{50} and LC_{90} if a dose–response relation is required. Survival analysis (Cox proportional hazards, Kaplan–Meier) may be appropriate to define the duration of effect.

4.5 SUMMARY OF EFFICACY INDICATORS FOR SMALL-SCALE AND SEMI-FIELD EVALUATIONS

The association between abundance and age structure of *Aedes* and disease transmission is not clearly defined and is likely to vary by ecological and epidemiological setting. Consequently, further evidence is required to set threshold values for the proportion of the *Aedes* population that a trap should remove in order to affect disease transmission

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Trap developers should seek to maximize efficacy in small-scale testing to ensure that the product has the highest possible mosquito catch rate in the field. For capture–kill traps, a consistent rate of 70% mortality or capture for the claimed duration of efficacy is desirable before proceeding with large-scale field testing (section 5). For autodissemination devices, guidance will be revised as further data are generated.

A candidate trap is assessed against its efficacy in semi-field or large cage tests for the following variables:

- adult mortality (immediate or delayed);
- larval mortality or EI;
- attraction-oviposition: trap oviposition rates (eggs in trap relative to control);
- auto-dissemination: percentage adult EI from secondary containers;
- duration of activity: number of days or weeks for which efficacy end-points meet product claims; and
- density of application: optimal number of traps per unit area.

5. LARGE-SCALE ENTOMOLOGICAL FIELD TRIALS OF VECTOR TRAPS

Candidate vector traps that are efficacious in small-scale field trials should be validated in large-scale entomological field trials against natural *Aedes* spp. populations. These trials are intended to demonstrate whether use of the traps over an area can control local populations of *Aedes* spp. and/or change the age composition of adult female mosquitos. The experimental design must be statistically robust and have the power to demonstrate a specified reduction or difference in key parameters between treatment and control clusters. The tests should also indicate the physical durability and attrition of traps, user acceptance and effects on non-target organisms.

Details of methods for planning and conducting entomological trials are beyond the scope of this document, and the WHO manual on study design of field trials for vector control interventions (*28*), other resources (e.g. *31, 32*) and a specialist in trial design and implementation for vector control should be consulted.

The objectives of such tests are to:

- confirm the efficacy and duration of the effect of traps to reduce vector populations and/or alter population structure under field conditions at the defined trap density;
- assess the physical durability and attrition of traps in field conditions;
- observe and record the ease of application, handling and perceived adverse effects during product application and use;
- for traps that include an insecticide component, determine insecticide resistance before and after the trial; and
- observe and record the effects on non-target organisms, including pests (e.g. *Culex* mosquitos) and beneficial insects (e.g. bees).

The design of large-scale entomological field trials must be robust and preferably be a cluster randomized trial that meets the criteria of replication, randomized trap allocation and adequate sample size. Vector traps in the treatment clusters are distributed at the intended density, coverage (i.e. number and placement of traps per unit area) and position inside and/or outside houses. Efficacy is assessed by comparing differences in vector population density and age structure (including sex ratio and parity) in the treatment and control (no traps) arms of the trial. Tests will also demonstrate the physical durability and attrition rates of traps, acceptability by users and effects on non-target organisms.

5.1. GENERAL CONSIDERATIONS FOR TESTING

The entomological outcomes of field trials on vector control interventions are specific for the setting in which the trial was conducted. Full assessment of the efficacy of candidate traps might require testing in several ecological settings and in different seasons, depending on the product claims. The area and location of trial sites should be representative of the target species' habitat and the expected conditions of human

exposure. Generally, at least two well-conducted large-scale field trials are required for a WHO policy recommendation.[1]

The WHO Vector Control Advisory Group recommends that first-in-class vector traps intended for public health initiatives be tested in trials that include epidemiological end-points (section 6), in addition to field trials with entomological end-points described here. Next-in-class traps do not need to show data on epidemiological efficacy and can be assessed from entomological data alone.

5.2.   ETHICAL CONSIDERATIONS AND COMMUNITY SENSITIZATION

Ethical approval should be received from the appropriate ethical committees before any trial procedures are started. The design of the study, participant information sheets and consent forms should undergo ethical review. Key considerations include: increased exposure to vector-borne diseases from additional aquatic sites or diversion of vectors, potential adverse effects associated with human exposure to the traps (as described by the manufacturer; see section 6) and effects on non-target organisms such as pollinators.

Human use protocols should clearly describe the potential risks associated with use of and exposure to the traps and strategies to mitigate such risks. Examples include: instructions for trap monitoring by project personnel and appropriate disposal (e.g. on completion of the study) to ensure that traps do not become larval habitats; exclusion criteria for households that cannot provide access for trap monitoring; and provision of clear descriptions of potential health risks to study participants when obtaining consent, including the contact details of study personnel and instructions for participants if they experience any physical symptoms associated with exposure to the traps. Households must be informed about the procedures and the frequency of monitoring visits associated with their participation in the trial.

Engagement strategies should include working with community leaders and members to inform them about the trial objectives. Informed consent must be obtained from individual households and/or the communities when appropriate. If trials are conducted in areas with possible virus transmission, control and treatment sites should continue to receive vector control according to the standard of care, including emergency control (e.g. space spraying) interventions. Coordination with local health authorities to keep the lines of communication open can mitigate the impact of these activities on trial results to ensure that all activities are properly documented and that all study clusters receive any emergency control measures equally.

Risk assessments that take into account the type of device, the attractant, the insecticide used and the environment in which the trap will be set may be required before testing, according to the protocols of the testing institutions. If during the field tests evidence arises that other insects (e.g. honey bees) are being collected or their populations reduced, further studies may be required to measure the impact.

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1.   Applicants are strongly encouraged to contact VCAG (vcag@who.int) to ensure that the appropriate procedures for generating evidence for public health are followed and to consult the latest guidance on its website and that of the WHO prequalification team for vector control products.

5.3.   STUDY DESIGN

WHO guidance on the design of phase 3 vector control field trials (*28*) should be referred to for additional guidance.

5.3.1 SAMPLE SIZE

Before a trial begins, the necessary sample size should be estimated to ensure that the trial has enough power to quantify the effectiveness of vector traps against the entomological indicators of interest (*33, 34*). Local heterogeneity in *Aedes* numbers may influence the sample size and number of replicates.

5.3.2 DURATION OF TRIAL

Entomological field trials should be conducted over a minimum of a full transmission season, as trap performance is likely to depend on mosquito density and environmental conditions. Baseline characterization of local field sites is recommended; the data collected can be used for stratification and allocation of treatment and control clusters. For more details of study designs that require baseline data collection, see Wilson et al. (*31*). The benefits of planning longer trials should be considered, to account for the risk that atypical meteorological events (e.g. hurricanes), political or civil unrest or disease outbreaks (*Aedes*-borne or other infections) will confound or disrupt the trial. For first-in-class products, where both entomological and epidemiological field trials are planned, VCAG recommends a 2-year trial duration, excluding baseline data collection, to generate data on the consistent entomological and epidemiological outcomes across consecutive high and low transmission seasons.

5.3.3 STUDY AREAS

Study sites should be carefully selected to ensure that treated areas and controls are independent but comparable (e.g. in terms of ecology, housing type, predominant larval habitats and meteorological conditions). Clusters should be of similar sizes, with a minimum size equal to the flight range of *Aedes* spp. (150–200 m radius, e.g. a few blocks in urban sites and a single village in rural sites). Clusters should be spatially separated and not contiguous or adjacent. A separation of two to three times the flight range of *Aedes* spp. is ideal (300–600 m).

If isolated areas cannot be used for testing, traps can be deployed over a sufficiently large experimental area so that entomological assessment can be restricted to a central zone where the impact of treatment will be greatest. Mosquito density just outside the experimental area can then be measured and compared with that in the centre of the treated area.

The environmental conditions of temperature, humidity, rainfall and wind speed should be monitored and reported during evaluations for multivariate analyses and to quantify trends. Ideally, environmental conditions should be monitored at several points in the trial site. If this is not possible, data may be collected from weather stations in the study area. If the device being tested includes insecticides, the insecticide resistance profile of the target species in the testing area should be considered (*15*).

5.3.4 PRE-INTERVENTION SITE CHARACTERIZATION

As trials must have comparable treatment and control areas, preliminary characterization of the areas is essential. The length of the survey will depend on the study design. Baseline surveys are conducted to characterize parameters such as vector abundance (section 5.3.6), insecticide resistance and housing and other relevant characteristics to control for underlying sources of variation in the analysis or stratify allocation on variables with wide variation among clusters. For designs that compare study areas before and after trap deployment, the pre-intervention survey should be long enough to capture temporal variation in the study area.

5.3.5 TRAP PLACEMENT

Trap density (per area or dwelling), coverage (percentage of area or dwellings with traps) and placement (preferred locations) are decided on the basis of manufacturers' recommendations and the evidence provided by the small-scale trials. The number of traps required per study area in each trial depends on the type of trap, the size of the trial area, the estimated area covered by traps and the estimated adult mosquito population density before trapping. At a minimum, an adequate trial should achieve 80% of the planned coverage (i.e. the predetermined number of traps required) in each study area; any shortfall in coverage should be recorded and reported. If the study design specifies that trap placement be accompanied by larval source reduction (e.g. removal of tyres and other secondary containers), similar source reduction should be undertaken in the control arms of the trial.

5.3.6 ADVERSE EFFECTS (SEE ALSO SECTION 6)

Adverse effects and events due to use of the trap product, general acceptance by local inhabitants and attrition (missing or destroyed traps) in the trial area should be observed and recorded, such as for instance records of people who did not accept to participate or dropped out and those who were retained (35). A GIS database may be useful for monitoring traps and trap attrition.

5.3.7 SAMPLING AND MONITORING

Ideally, more than one monitoring method should be used for assessing effects on *Aedes* populations or mosquito survival. Sampling schemes (number of days sampled per week) should be standardized for all study areas. For interventions targeting *Ae. aegypti*, sampling should be conducted in or around households. *Ae. albopictus* is found in a wider range of habitats both near and far from human population centres (urban, rural and forested). Methods for sampling should be evaluated under local conditions before use and with consideration of the local ecology of the target vector. If traps are used for monitoring in field trials, these should be placed at a distance far enough from the intervention trap that there is no competition between the two (e.g. not in the same household or room).

The recommended sampling methods are adult aspiration for *Ae. aegypti* (e.g. CDC Backpack, Prokopack) and traps for *Aedes* surveillance (e.g. BG Sentinel traps, autocidal gravid ovitrap, gravid *Aedes* trap, infusion-baited ovitraps) (*36*) (**Fig. 3**).

Fig. 3. Common sampling devices for *Aedes* spp. mosquitos. (trap images adapted from reference *36*)

| BG-Sentinel 2 | Autocidal gravid ovitrap | Gravid *Aedes* trap | Prokopack |

Larval or pupal surveys provide valuable supplementary information on *Aedes* ecology in study areas, but indices for such immature stages should be considered secondary measures. The presence of eggs in ovitraps can indicate the presence or absence of *Aedes* spp. and is used in many programmes; however, because the density of both larvae and eggs in ovitraps depends on the availability of containers and is not necessarily directly related to changes in adult density, this measure is not recommended for assessing the effect of vector traps on populations.

Human landing collection of *Aedes* mosquitoes is not recommended where there is the risk of exposure of field collectors to arbovirus and the lack of prophylaxis for *Aedes*-borne diseases. Some researchers have used double nets or electrified nets to collect *Aedes* mosquitos in the field, thus preventing human baits from being bitten (*41,42*). Sweep net collections have been used for collecting adult *Ae. albopictus* (*43*).

Methods for surveillance of *Aedes* mosquitos have been described comprehensively elsewhere (*4, 36, 44*), including the use of infusion-baited ovitraps.

5.4. MEASURING EFFICACY OF TRAPS AGAINST ENTOMOLOGICAL END-POINTS

The objective of entomological evaluations is to determine whether the adult female *Aedes* population or mosquito survival is reduced significantly by the vector trap intervention. To determine the effect of traps on the target vector population, adult densities and age structure should be evaluated by collecting samples in treatment and control areas by the same standardized sampling scheme used for baseline characterization of the site. Sampling should be frequent enough to account for temporal and spatial variation in the mosquito population throughout the trial. For guidance, sampling intervals of 1–3 weeks should be used.

5.4.1 ASSESSMENT OF ADULT POPULATION DENSITY

Adult mosquito densities in and around houses in treatment and control areas can be monitored at fixed trapping points (in adult traps) or in house-to-house surveys (by aspiration). House-to-house surveys cover more houses per unit time and ensure better spatial coverage than fixed traps, but they are labour-intensive and depend strongly on the skill and diligence of the operator. Fixed trap methods better capture short-term temporal variation.

Sampling procedures should be standardized as far as possible to maximize consistency in the results. Detailed procedures for household surveys with aspirators are provided in the WHO guidelines on evaluation of space sprays (*45*). The aim of the procedures is to sample the adult vector population in the study areas reliably, as expressed by the average number of mosquitos per room, per house or per other defined unit sampling point.

5.4.2 ASSESSMENT OF MOSQUITO POPULATION STRUCTURE AND PHYSIOLOGY

The age structure of the mosquito population in the field can be estimated from the frequency of nulliparous and parous mosquitos. The proportion of parous females is an indirect measure of the probability of daily survival of mosquitos in the population. Parity is a useful indicator in mosquito populations that are stable over time, as demonstrated by surveillance in the study area, for example during site characterization before the intervention.

5.4.3 AUTODISSEMINATION EFFICACY

While the aim of large-scale entomological field trials is to detect entomological effects on the population due to the presence of vector traps, for traps that function by autodissemination, it may also be useful to monitor the efficacy of autodissemination over time. Autodissemination monitoring ovicups (*46*) or larval bioassays in water sampled from natural aquatic habitats (i.e. water bodies with *Aedes* larvae) can be used to measure autodissemination efficacy. Laboratory-reared *Aedes* larvae added to these samples and *Aedes* larvae collected from natural sites are monitored for emergence inhibition. For ovicup monitoring, a trap:ovicup ratio of no more than 1:5 is recommended to avoid an effect of ovicups on the overall mosquito population. Autodissemination efficacy may increase with time due accumulation of the autodisseminant (e.g. pyriproxifen) from multiple visits of mosquitos to the oviposition site or ovicup.

5.5. PHYSICAL INTEGRITY AND DURATION OF EFFECT

Most traps require periodic servicing or maintenance. Trap durability and efficacy should be assessed during the servicing interval (i.e. the time in days or months for which products are effective without servicing), and a longer-term assessment should be done to determine trap integrity and retention or loss and to confirm the duration of trap efficacy. The duration of each study should be appropriate for validating the manufacturers' claims. During servicing, physical integrity and trap presence should be recorded, and quality assurance assays can be conducted on certain components of the traps. Alternatively, assessments can be made of manufacturers' claim by simple random sampling of traps in the study area.

A standard sampling questionnaire should be used to collect data on the integrity, durability and attrition of traps. Mobile devices and GIS databases may be helpful for data collection and tracking and should be explored. The aspects listed below should be investigated.

- Physical integrity: A standardized form should be prepared for recording the general condition of the trap, including (where relevant) condition of insecticide components or adhesive strips (e.g. presence, whether torn or have holes), water levels, presence of larvicide or attractant.
- Trap functionality: presence of adult and immature mosquitos and other insects.
- Quality assurance of trap components (see section 3.5): bioassays with insecticide-treated materials in traps, assessment of adhesives and evaluation of larvicidal activity.
- Trap attrition: whether traps have been lost or moved, whether residents have washed or modified the traps against study instructions.
- Household retention: withdrawals and coverage rates.

5.6. OBSERVED NON-TARGET EFFECTS

Candidate traps tested under field conditions must be assessed for ecological and human toxicity before a field study is conducted. Detailed treatment and analysis of these data are beyond the scope of this document; however, during large-scale trials, when appropriate, qualitative observations should be recorded on non-target species that are protected or would affect allied species such as bees and other pollinators (*47*). For example, non-target organisms found in traps or any noticeable impact on cohabiting organisms found during larval sampling (e.g. fish, copepods, other mosquito larvae) could be noted.

5.7.   EFFICACY INDICATORS FOR ENTOMOLOGICAL FIELD TRIALS

A candidate trap, with bait and/or insecticide, is tested for efficacy in large-scale entomological trials against the following primary criteria:

- local adult *Aedes* mosquito population density: significant difference in mosquito population density between treated and control areas;
- local adult *Aedes* mosquito population structure: significant decrease in the proportion of older female (parous) mosquitos.

The following secondary indicators support efficacy assessments, and, when possible, the results should be reported.

- sex ratio shift: a significant increase in the proportion of males in the treated area;
- oviposition rates: significant decrease in mean egg catch in the treated area;
- physiological status: significant decrease in the number of blood-fed females collected in the treated area; and
- infection rate: proportion of vectors infected (see section 6.3).

6. COMMUNITY TRIALS OF IMPACT ON DISEASE

The public health effect of first-in-class vector traps against natural vector populations is assessed in community trials of the epidemiological impact on the incidence of *Aedes*-borne virus (ABV) or *Aedes*-borne disease in study clusters with and without traps.

Before traps can be recommended for public health programmes, evidence is required to support the principle that a vector trap strategy can reduce infection and/or disease. To that end, the Vector Control Advisory Group recommends that at least two well-implemented, randomized, controlled trials be conducted of epidemiological outcomes in different eco-epidemiological settings for a full assessment of the public health value (i.e. reduction of infection and/or disease) of this intervention strategy (*48*). The duration of epidemiological assessment, excluding the baseline period, should cover at least 2 years, to account for inter-annual variation in transmission. Individual next-in-line traps may not require such evidence, and applicants are strongly encouraged to contact the relevant WHO programmes (i.e. VCAG and PQT-VC) to ensure that the appropriate evidence is generated.

Large-scale entomological field trials are described in the previous section. As detailed methods for planning and conducting epidemiological trials are beyond the scope of this document, the WHO manual on study design of field trials for vector control interventions (*28*), other resources (e.g. *31, 32*) and a specialist in epidemiological trial design and implementation for vector control should be consulted.

Study designs are affected by conditions that are impossible to control, including household access and coverage, heterogeneous housing, movement of people, security issues and other public health programme activities, as well as unpredictable virus transmission dynamics. Accurate evaluation of interventions requires a robust study design.

The objectives of a community trial are to:

- demonstrate the protective efficacy of traps for ABV transmission and/or *Aedes*-borne disease incidence;
- monitor severe and adverse events in the human population; and
- observe and record acceptability, coverage and maintenance during product application and use (the trap itself and the bait and/or insecticide), ease of application and handling, associated costs and any consequences associated with maintenance failure (trap loss and conversion into a larval habitat).

In this section, we describe measurement of virus transmission, disease and related proxies, ethical considerations, human safety, blinding and trap effectiveness.

6.1. MEASURING TRAP EFFICACY AGAINST EPIDEMIOLOGICAL END-POINTS

The primary epidemiological end-point is demonstration of the protective efficacy of the trap intervention. As an expected rate of protective efficacy is required for calculating sample size, a minimum of 30% is recommended.[1] Entomological end-points should be consistent with the mode of action of the traps (see section 5). Defining strategies for monitoring virus transmission or disease in human populations is particularly challenging for *Aedes*-borne diseases. In these guidelines, we focus on diseases caused by dengue, Zika and chikungunya viruses. Dengue and Zika viruses are in the family *Flaviviridae*, whereas chikungunya virus is in the family *Alphaviridae*.

During the first 5 days of acute infection, virus can be detected by cell culture or polymerase chain reaction (PCR) of key RNA sequences. Effective disease surveillance systems and confirmatory laboratory diagnostic capacity are required to identify and test potentially infected individuals. When possible, several strategies should be used to measure infection (section 6.1.2) and/or disease (section 6.1.3) in community-based trials. All residents in the study area can be monitored for disease. For infection, a subset of residents most likely to be susceptible (e.g. children) is identified during the baseline screening study. Blood samples from these individuals are tested at regular intervals to monitor seroincidence.

Monitoring multiple epidemiological parameters will increase the probability of detecting PE if an intervention is effective, but monitoring both disease and infection is not a requirement and may not be feasible or appropriate in certain locations. PE can be sufficiently demonstrated with a single epidemiological endpoint.

6.1.1 BASELINE AND SCREENING STUDIES

As *Aedes*-borne viruses cause "sterilizing immunity" to the infecting virus serotype, the age-specific seroprevalence of arbovirus serotypes in the study population should be known to understand heterogeneity among clusters and to stratify the allocation of traps. Residents in the study cluster(s) should be screened to determine prior ABV exposure, and only those showing negative or monotypic ABV response should be included as participants in the sero-incidence studies (section 6.1.2). Because of significant cross-reactivity in diagnostic tests between dengue serotypes and Zika virus infections, inclusion of participants with a multi-typic response is not recommended.

The serological status of study residents is used to identify a longitudinal cohort (see section 6.1.2) and to characterize the susceptibility of the human population in each study cluster to ABV infection. This information can be used to stratify clusters before trap allocation (e.g. clusters can be stratified into high, medium and low seroprevalence groups), and allocation to treatment and control be balanced within each stratum. Serology is the method used to detect ABV infection after the acute phase of infection is over. Plaque reduction neutralization or microneutralization assays should be performed,

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1. The value of 30% protective efficacy was intended as a conservative, practically achievable rate of protection, as recommended during expert consultation and a review of similar efficacy trials of targeted interventions against *Aedes*.

when possible, to determine serotype specificity. Alternatively, an immunoglobulin G enzyme-linked immunosorbent assay (IgG ELISA) can be used to distinguish between naive and previously exposed individuals, although this assay is not serotype-specific. **Fig. 4** summarizes the timelines for antibody responses and detection methods after dengue infection. The WHO guidelines on dengue diagnosis, treatment, prevention and control (36) should be consulted for details and resources.

Fig. 4. Approximate timelines of primary and secondary dengue virus infections and methods that can be used to detect infection
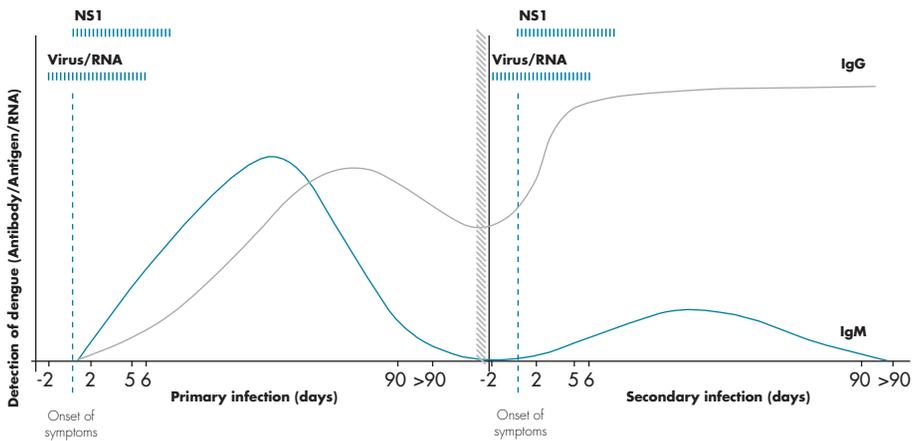
Adapted from the WHO guidelines for diagnosis, treatment, prevention and control of dengue (*36*) and www.cdc.gov/dengue/clinicalLab/laboratory.html

IgM, immunoglobulin M; NS1, nonstructural protein 1

6.1.2 SEROINCIDENCE STUDIES

A subset of the population in the study clusters should be recruited for longitudinal blood sampling. Blood samples collected at 6–12-month intervals from the same individuals are tested for virus in plaque reduction neutralization or microneutralization assays, as described above for baseline surveys. Ideally, the cohort members should have had no prior ABV infection, exception for dengue virus, when inclusion of individuals who have been exposed to a single virus serotype would be appropriate. Studies suggest that in most dengue-endemic regions > 90% of adults have had at least one dengue infection. Therefore, paediatric cohorts are recommended, as the risk of infection increases with time.

When an individual has antibody titres above the threshold level after a previously negative blood sample, he or she is assumed to have developed an infection during the time between the two samples. Individuals who show no change in antibody titres are assumed not to have seroconverted. Seroincidence rates can be calculated by cluster and time interval from all individuals who provide paired blood samples:

Seroincidence rate = number of seroconversions / sum person-time.

Alternatives to this approach include IgG ELISA or haemagglutination inhibition assays on blood samples taken at 3–6-month intervals to identify seroconversion. IgG ELISA of saliva samples has been used as a proxy to identify dengue virus transmission, but positive samples do not necessarily represent new infections (*49*).

6.1.3 DISEASE SURVEILLANCE

A second strategy for measuring public health impact is quantification of cases of *Aedes*-borne disease within study clusters. Study participants can be instructed to present to local health facilities when they have symptoms, or an active disease surveillance system can be set up. Historically, fever has been a clear trigger for either presenting to a health facility or as a key criterion for identifying individuals to be screened for ABV. As many cases of Zika virus disease do not present with fever, study participants can be told to watch for rash and/or fever accompanied by joint pain and/or red eyes (50). It is critical that the surveillance protocol and case inclusion criteria be consistent for all study clusters throughout the study.

Blood samples from both acute and convalescent cases should be obtained for laboratory diagnosis. Samples taken during the first 5 days of illness should be tested by PCR or nonstructural protein 1 (NS1) testing. When individuals present with clinical symptoms but test negative by PCR, a further blood sample should be taken 14–21 days later to test for virus-specific IgM or IgG antibodies (**Fig. 4**).

In all trials, disease surveillance protocols must be consistent and the population participating in surveillance be well characterized. The most commonly used disease surveillance strategies are listed below.

Passive surveillance in health facilities or by study personnel

Study participants can be given clear instructions to notify study personnel or to present to a designated local study clinic if they have fever or other specific symptoms. Usually, they are given a card that identifies them as study participants and provides contact information. This strategy works well if access to facilities or study personnel is readily available. As the method relies on the initiative of study participants, it can be improved by periodic phone calls or reminders. Health-seeking behaviour varies, especially by age. Passive case-finding should be considered a complementary outcome, and active surveillance is preferred to avoid treatment-seeking bias.

Surveillance in schools or workplaces

Absence from school or work has been used as a trigger for visiting study participants and obtaining samples for diagnosis if the absence is due to illness. Although this system may be effective for epidemiological studies, it is not a recommended strategy unless the experimental units are schools or workplaces. This type of surveillance may reveal cases, but it greatly restricts the size of the surveillance cohort. For community interventions, surveillance at the household level conducted by active house visits is preferred.

Active house visits

Households in the study clusters may be visited once to three times a week to ask whether individuals have fever or other symptoms. Although this system is labour-intensive, it is the most sensitive approach for identifying potential cases. Furthermore, individuals who are ill who do not agree to provide samples can be counted to identify potential participation bias in clusters.

Household census (denominator)

Calculation of seroincidence rates requires reliable, precise estimates of the number of individuals under surveillance. This requires household censuses and monitoring of residents' movements in and outside households to document their presence and absence in the study area. Census information must therefore be updated periodically.

Time in house (exposure)

As traps are deployed at cluster level, additional studies are required to determine the proportion of time individuals in the population under surveillance are exposed to the vector control intervention at both household and cluster level. This information can be collected through interviews or methods such as GPS tracking. Both seroconversion and seroincidence calculations will have to be adjusted to person–time data to account for time not exposed to the intervention.

6.1.4 CROSS-SECTIONAL SURVEYS

During periods of very high transmission, a series of standardized cross-sectional surveys across clusters could be used to identify infected people in order to determine the public health impact of an intervention. Although this strategy is not recommended as the primary or only epidemiological method, it would be appropriate after the introduction of a novel virus or serotype into a study area (for example, during periods of epidemic transmission). To increase the probability of detecting a significant public health impact, a protocol including sample size calculations could be prepared in advance for use in the case of an outbreak. If high rates of ABV infection are documented, the duration of the trial could be shortened. A random selection of individuals in each cluster under surveillance would provide blood samples each month to be tested for evidence of acute infection (PCR, IgM and NS1).

6.1.5 CHANGES IN MOSQUITO INFECTION RATE

As part of entomological monitoring in a trial, adult female mosquitos may be tested for ABV by PCR or NS1. Mosquitos that have had recent blood meals should be tested separately from those without evidence of a recent feeding and from gravid mosquitos. All species of mosquito, including abundant *Culex* mosquitos, with recent blood meals can be tested for ABV, as these will test positive if they have recently fed on an infected person even if they are not vectors of the disease. Positivity in gravid and non-gravid *Ae. aegypti* (or other known *Aedes*) females is used to estimate the number of infectious mosquitos in each cluster. The mosquito infection rate is potentially a proxy for human infection. At present this would be an appropriate secondary outcome, but it cannot substitute for seroconversion or disease incidence.

6.1.6 BLINDING

To reduce potential study bias, blinding to the intervention is usually recommended in clinical trials. When traps are used, blinding of study participants and field staff may not be practical; however, measures should be in place to ensure blinding of laboratory data, both virological and entomological, as well as data management and analysis. A standard of care alternative, such as larviciding, is recommended for comparison in all study clusters, both intervention and control. Equal, standardized treatment must be used in all study clusters for disease surveillance. Mock trap devices (with no water, no insecticide, easy escape) could be used; however, this approach is limited because participants must have information on trap components and their risks before they provide informed consent.

Teams responsible for different components of the study (disease monitoring, entomological monitoring, trap deployment and maintenance, laboratory) should work independently to avoid unintentional bias. For example, different teams should be responsible for implementation and for evaluation.

6.2. ETHICAL CONSIDERATIONS, STUDY REGISTRATION AND MONITORING

Full ethical considerations are not covered in this document, and appropriate sources and experts should be consulted during the planning of trials. Guidance on the ethical design and conduct of cluster randomized trials is provided in the Ottawa Statement (*51*).

6.2.1 STUDY REGISTRATION

It is strongly recommended that community trials (randomized controlled trials) be registered as clinical trials in an appropriate registry before they are initiated. This step has a number of important implications: (i) compliance with local regulatory institutions by passing all protocols through national institutional review boards responsible for clinical trials; (ii) a clear plan for allocation of the intervention, including a method for

generating an allocation sequence, a list of the factors used for stratification and a method for implementation; (iii) a clear statement of who will be blinded (participants, study personnel, data analysts) and how; (iv) data monitoring and audits; and (v) monitoring of safety and structures for implementation, e.g. a data safety monitoring board. In addition, it is best practice to have documented procedures (standard operating procedures) for all aspects of trial conduct and data collection, e.g. for procedures such as drawing blood, trap deployment, mosquito collection and data management.

6.2.2 MONITORING OF ADVERSE EVENTS

Although most anticipated trap designs are not expected to be associated with more than minimal risk, many contain chemical insecticides or parts that could be ingested or cause allergic or physical reactions on physical contact. Severe adverse events must be distinguished from expected minor-to-moderate adverse events described in the manufacturer's brochure.

Examples of severe adverse event include death or severe injury after choking on a trap component, asthma requiring hospitalization induced by exposure to a chemical component of a trap or serious injury due to tripping over a trap. The rules for reporting severe adverse events depend on the institutional review board or ethics committee; however, a severe adverse event that is likely or potentially to be attributable to the intervention must be reported within 24 h and be reported formally within 5 working days (these times might vary). Severe adverse events that are not likely to be associated with the intervention should also be reported to institutional review boards and to data and safety monitoring boards under their defined conditions (annually or quarterly). The events will be analysed by these independent boards for any unusual patterns or unexpected association with the intervention.

A critical component of a community trial is quantification of adverse effects of special interest. Examples include mild skin or eye irritation after contact with the trap, allergic reactions or increased symptoms of mild asthma. Unexpected adverse events, even if they are not severe, must be reported promptly. Clear reporting and recording protocols are required for complaints from participants about such events to study personnel. If possible, complaints should be followed up by study medical personnel for better characterization. Study databases should include tables for recording events linked to affected participants. As many such events are mild, participants may not report them to study personnel; therefore, at the time of consent, expected adverse events should be described and participants encouraged to report them to study personnel. Further, when participants withdraw from a study, they should be asked about the occurrence of adverse events and whether they were a factor in their decision to withdraw. Separate questionnaires or a complement to disease surveillance could also be used. In all cases, it is important not to introduce bias or potentially unblind studies.

6.2.3 INDEPENDENT MONITORING

It is recommended that independent entities be engaged through a contract research organization to monitor trials, such as a data and safety monitoring board for adverse events and independent quality assurance.

6.3. TRAP MONITORING, MAINTENANCE, COVERAGE AND SCALE-UP

The objective of the community trials is to test traps under the most closely controlled conditions possible. This often requires that trap maintenance be managed by study personnel, which may not be feasible in national vector control programmes. We recommend, if possible, use of pilot studies to examine how the community or the programme staff will be involved in trap maintenance.

6.3.1 TRAP MAINTENANCE

Trap specifications must be clearly defined, including the requirements for their use (addition of water, baits) and frequency of maintenance (cleaning and/or recharge). Compliance with these specifications should be monitored and recorded, as should movement and alteration of traps and those that are no longer effective, for example traps that have been tipped over or emptied and then returned to their position without larvicide.

6.3.2 TRAP COVERAGE

Coverage must be monitored throughout the trial, including the proportion of lots (housing and other) with traps; the proportion of lots with traps in place, functioning as planned and cleaned or recharged successfully; and traps that have disappeared and households that withdraw from the study. A monitoring system should be in place that tracks individual traps.

Although the coverage required for a public health impact is unknown, it is recommended that studies maintain 80% of the planned coverage. For example, for area-wide protection, the aim would be to include at least 80% of the planned houses or properties in the study area. Importantly, for the households participating in disease monitoring, studies should demonstrate that traps were in place and properly maintained 80% of the time and that at least 80% of the households were retained for the duration of the study. It may be difficult to achieve this proportion in field trials. The total numbers of traps, participating households and properly maintained traps must be recorded throughout the study (**Box 1**).

Box 1. EXAMPLE OF CALCULATING AND MAINTAINING TRAP COVERAGE

Initial coverage

In a study with a lethal ovitrap that requires that the larvicide component be changed every month and an optimal density of three traps per property in a study cluster of 100 houses, the cluster should have 300 traps, with three on each property. After initial deployment, spatial coverage can be calculated from:

number of houses with traps / number of houses in the cluster.

For example, if 80 of 100 household accepted traps, coverage would be 80%; alternatively, 240 of 300 traps, would be 82% coverage.

Follow-up

Each household would be visited monthly for 1 year. For the 80 participating houses, the larvicide would have to be changed 960 times. As some people might not be at home, in this example there should be a minimum of 768 successful visits (768/960 = 80%).

Some households withdraw or traps are lost. For example, if 10 household withdraw, the coverage rate would drop to 70%. Coverage should be monitored by cluster and at each appropriate monitoring visit.

Houses may have damaged traps. If 50/80 houses lose one of three traps, (30 x 3) + (50 x 2) = 190 traps would remain (63% coverage). Trap density during follow-up should therefore also be calculated.

6.3.3 SCALING-UP TRAP INTERVENTIONS

Extending the use of vector traps may require a wide array of measures that are not included in this document. One issue relevant to traps is ensuring distribution and maintenance. Distribution schemes should be tested in effectiveness trials, with coverage as the relevant end-point. Consideration should be given to trap maintenance (ideally by the community), monitoring and evaluation procedures and plans for disposing of used and unused traps.

6.3.4 COMMUNITY PERCEPTIONS AND ACCEPTANCE

A social component to assess communities' reaction to the intervention should be included. A variety of qualitative research techniques are available, such as focus group discussions and key informant interviews. Additionally, periodic quantitative surveys should be carried out of community perceptions about the acceptability and efficacy of the traps.

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SUPPLEMENTAL MATERIALS

S1. SAMPLE PROCEDURE FOR EVALUATING AUTODISSEMINATION AGENTS

Autodissemination is the ability of adult mosquitos to pick up a contaminant from treated solid surfaces and to retain and transfer it to aquatic habitats in sufficient quantities to contaminate the habitats, rendering them unproductive, either by killing larvae or preventing pupae from emerging to adults.

The aim of this assay is to establish the dose–response relation of the autodisseminant on the adult mosquitos to achieve 50% and 90% mortality of susceptible mosquito larvae that are exposed by transfer of the autodisseminant from the adult to the larval habitat. The protocols are adapted from Sihuincha et al. (*1*), Lwetoijera et al. (*2*) and WHO (*3*); however, further independent validation of this assay may be needed.

Mosquito species and test conditions

Tests should be conducted on well-characterized, strains of mosquito that are susceptible to all major insecticide classes with no detectable resistance mechanisms, reared according to standard institutional protocols (e.g. 27 °C ± 2 °C, 80% ± 10% relative humidity and photoperiod 12 h light : 12 h dark). For autodissemination experiments, blood-fed and gravid mosquitos (e.g. 6–8-day-old females that took their first blood meal 2–4 days before the experiments) on sugar meals (e.g. 10% sucrose) should be used.

Methods

A modified bottle bioassay (*4*) is used for testing active ingredients (AIs) for autodissemination. In this assay, 1 mL of a solution of either the carrier or solvent alone (e.g. acetone) or of the desired concentration of insecticide in the same carrier or solvent is placed in a 250-mL glass bottle (e.g. Wheaton®). Dilutions of AIs should represent five to six test concentrations that cause 0–100% inhibition of emergence of larvae. A minimum of four replicates of each serial concentration and two control bottles (solvent only) should be prepared.

Groups of 5 female mosquitos are added to each bottle and exposed for 30 min and 1 h. Control mosquitos are maintained in bottles containing only the solvent for 1 h. The bottles are turned every 15 min to maximize the chances that the mosquitos will pick up the candidate autodisseminant.

After exposure, the mosquitos are removed from each bottle and transferred to screened cages with bioassay containers (*3*) containing 200 mL water and 25 late-stage L3 / early L4 *Ae. aegypti* larvae with a larval food source. The containers are lined with filter paper as a substrate for oviposition, and mosquitos provided with access to 10% sugar solution. After a specified time (e.g. 24 h), adult mosquitos are removed, and mortality and inhibition of larval emergence are monitored in standard larval bioassays (*3*).

If adult emergence in the controls is < 80%, the test should be discarded and repeated. If the percentage in controls is 80–95%, the data may be corrected with Abbott's formula. Cumulative totals of dead larvae and pupae from each assay are pooled for dose–response analysis by probit analysis.

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S2. SAMPLE DATA REPORTING FORM FOR SMALL-SCALE TRIALS

Date/time start:_____   Temperature/relative humidity: _____   Location: _____

Time stop: _____   Temperature/relative humidity: _____

Test item:_____   Control 1: _____   Control (If positive standard used): _____

Test system/strain: _____   Mosquito age: _____   Time blood-fed: _____

Number replicates: _____   Mosquitos released/cage: 1_____ 2_____ 3_____ 4_____

Cage	Trap location						No. alive	No. dead	Total recovered
	A	B	C	D	E	F			
1 Treatment									
No. trapped/ No. eggs									
2 Treatment									
No. trapped/ No. eggs									
3 Treatment									
No. trapped/ No. eggs									
4 Treatment									
No. trapped/ No. eggs									

Specificities of attractant (if any): _____

Collector(s): _____   Notes:

Data recorded by: _____

Control mortality: _____   Acceptable range is < 10% for adulticide

New tools to target and suppress *Aedes* populations are needed to protect people living in areas of risk for arboviral disease. The purpose of this document is to provide procedures and criteria for testing the efficacy of and evaluating vector traps for disease control. It includes the design of laboratory and small-scale field trials to assess the attraction and killing effects of vector traps and of large-scale community trials to determine the efficacy of traps in reducing mosquito populations in the field and disease transmission. This document is intended to support product developers, programmes and testing institutions generate robust entomological evidence of the efficacy of vector traps for control and, for a first-in-class vector trap, evidence of the public health impact in reducing arboviral disease.