Considerations for the selection of mosquito species and strains for use in free-flight room studies and site selection for semi-field studies

Key factors to consider when selecting vector species/strains for use in free-flight room studies and which may affect validity of tests and studies:

- Vector species and strains for use in free-flight room studies and local vector populations at semi-field study sites should carry characteristics that are impacted by the active ingredient(s) (AIs) in the spatial emanator, and which align with the intended effect of the product.
- Diversity and variety in species/strain selection may provide information on the breadth of the spectrum of vectors against which the product may be efficacious.

1. Key principles

In planning for the generation of data to support an application for prequalification assessment of a spatial emanator product, manufacturers should consider:

- the selection of mosquito species and strains for use in **free-flight room studies**, and
- the composition of mosquito populations, including the local species characteristics, in the selection of sites for **semi-field** studies.

The species/strain selections must be appropriate for the evaluation of the intended entomological effects of the proposed spatial emanator product. The use of inappropriate species/strains may limit the usefulness of submitted studies, thereby diminishing the value of such studies in the decision-making process.

In free-flight room studies, the inclusion of a variety of species/strains across the supporting information is necessary to investigate, characterize and/or determine properties of the proposed spatial emanator product as well as demonstrate expectations of product performance across a range of target vectors. In semi-field studies, the requirement for submission of three semi-field studies, situated in locations where the characteristics of the local vector population are appropriate for the evaluation of the intended entomological effects of the proposed product, allows for the demonstration of product performance in a variety of settings.

The matrix of selected mosquito strains (MSMS) is a tool for identifying the characteristics of those mosquito species/strains with which data have been generated in flight room and semi-field studies.

Key considerations for species selection:

- Species must be known vectors of the disease for which the product is intended to provide protection.

2. Considerations for species and strain selection

2.1. Free-flight room studies

The conducting of the free-flight room study at the same testing facility where one of the semi-field studies is conducted allows for coherency in datasets and aids data interpretation.

2.1.1. Laboratory strains

In the process of developing product testing plans and generating supporting data for inclusion in a dossier for WHO prequalification assessment, manufacturers, and their associated partners, will need to consider the variety of species/strains of mosquitoes with which tests should be conducted.

Diversity and variety in species/strain selection may provide information on the breadth of the spectrum of vectors against which the product may be efficacious. Strain characteristics should be indicative of the target populations against which the product is intended to have an effect.

It is crucial to ensure that the characteristics of the selected mosquito strain(s) for use in free-flight room studies are such that the entomological mode of action (MOA) and the intended effects of the spatial emanator product can be demonstrated. The target characteristics of selected strains may be associated with insecticide resistance status or other biological characteristics that are not associated with resistance status. Spatial emanator products may therefore target multiple vector characteristics. Once the intended use of the product has been defined, the desired characteristics of laboratory strains and local vector populations can be specified.

2.1.2. Selection of species and strains

Free-flight room studies should be conducted using at least three mosquito species (*Aedes, Culex, Anopheles*). At least one insecticide-susceptible strain and one insecticide-resistant strain from each tested species should be used in the study. Thus, a standard free-flight room study will use six test system strains (Table 1).

The selected test system strains should be laboratory strains that are profiled for insecticide susceptibility status every six months using appropriate methods. Detailed documentation of the profiling schedule and results should be maintained by the responsible organization(s). Profile summaries should be appended to the MSMS.

Table 1. Test system species and strain requirements for free-flight room studies

Test system species	Requirements	
	Insecticide susceptible strain	Insecticide-resistant strain
Aedes	X	X
Culex	X	X
Anopheles	X	X

2.1.3. Identification and selection of insecticide-resistant strains or strains carrying desired non-insecticide-driven characteristics

In the early stages of product development, the target vector population(s) for the proposed spatial emanator product should be considered. This identification should include all vector populations against which the spatial emanator product is intended to be effective. For example, a spatial emanator product that contains a pyrethroid insecticide and a synergist will be designed to target pyrethroid-resistant mosquitoes carrying metabolic resistance mechanisms (primary target), but it will also have an impact on mosquitoes that are pyrethroid-susceptible (secondary target) and may, depending on the bioactive concentration of the pyrethroid, be partially effective against pyrethroid-resistant mosquitoes that have low or moderate levels of resistance expressed by non-metabolic resistance mechanisms (secondary target). It is possible to have multiple primary and secondary targets.

2.2. Considerations for study site selection for semi-field studies

Manufacturers should consider the composition of mosquito populations, including local species characteristics, in the selection of sites for semi-field studies. The vector population at selected sites should exhibit traits in alignment with the defined primary target(s) based on the MOA of the AI(s) and intended effects of the product. To assist with semi-field study site selection, characterization data for the vector population's target traits, for example, WHO susceptibility tests, insecticide resistance intensity assays, genomic screening, etc., generated by the study site should be considered.

Additionally, manufacturers should consider the National Regulatory Authority requirements for product registration in order to prioritize generation of efficacy data which can be used to support registration and/or selection decisions across multiple countries/organizations.

At least three sites in geographically distinct locations should be selected for semi-field studies.

3. Characterization of test system strains used in free-flight room studies and of local vector populations at semi-field sites

Characterization of selected strains for free-flight room studies and wild populations at semi-field sites should be conducted no more than six months before studies are initiated and should be concluded by the commencement of the study. Characterization data should include resistance intensity/mechanisms where insecticide resistance is a primary characteristic and tests for any traits that might cross-react with the proposed product's MOA, for example, cross-class resistance.

3.1. Methods for insecticide resistance characterization

Insecticide resistance status and intensity for the characterization of laboratory strains and local vector populations at semi-field sites should be conducted using the methods described in the *WHO manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions (1)*. Characterization results are recorded in the MSMS. At minimum, phenotypic resistance data must be provided. Genotypic characterization should be conducted where appropriate and appended to the MSMS.

Data from the characterization of the intensity of insecticide resistance is particularly useful to assist in interpretating results across semi-field studies, where diversity in mosquito vector populations may lead to differing impacts of the proposed product.

4. Related documents

- WHO PQT/VCP Implementation guidance – Free-flight room studies
- WHO PQT/VCP Implementation guidance – Semi-field studies for spatial emanator products
- WHO PQT/VCP Implementation guidance – Semi-field methods for spatial emanator products – Experimental hut tests
- WHO PQT/VCP Implementation guidance – Matrix of selected mosquito strains
- WHO PQT/VCP Implementation guidance – Template MSMS

5. References

1. Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. Geneva: World Health Organization; 2022 (https://iris.who.int/bitstream/handle/10665/356964/9789240051089-eng.pdf?sequence=1, accessed 20 June 2025).