WHO Prequalification of Vector Control Products

Considerations for the selection of mosquito strains for use in bioassays and site selection for semi-field and community studies

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

- Vector strains for use in bioassays, and local vector populations at semi-field study sites, should carry characteristics that are impacted by the active ingredient(s) (AI) on the insecticide-treated net (ITN), 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.
- Supplementary bioassays conducted as part of semi-field studies provide data regarding the consistency of the fabric of the ITNs used in the semi-field study and the consistency of sample preparation methods.

1. Key principles

In planning for the generation of data to support an application for prequalification assessment of an ITN, manufacturers should consider:

- the selection of mosquito species and strains for use in **bioassays**
- the composition of mosquito populations, including the local species/strain 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 product under investigation. 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 bioassays, inclusion of a variety of species/strains across the supporting information may be necessary to investigate, characterize and/or determine properties of a proposed ITN 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 product under investigation, 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 using bioassays and/or as part of semi-field studies.

2. Considerations

2.1. 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.

Key considerations for species/strain selection:

- Species must be known vectors of the disease for which the product is intended to provide protection.
- Strain characteristics should be indicative of the target populations against which the product is intended to have an effect.
- 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.

It is crucial to ensure that the characteristics of the selected mosquito strain(s) for use in laboratory bioassays and supplemental bioassays to semi-field studies are such that the entomological mode of action (MOA) and the intended effects of an ITN can be demonstrated. The target characteristics of selected strains may be associated with insecticide resistance status (for example, phenotypic, *i.e.,* insecticide susceptible or resistant, or genotypic, i.e. carriage of metabolic resistance mechanisms to pyrethroid insecticides), or other biological characteristics that are not associated with resistance status. ITNs that are treated with more than one active ingredient 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.

2.2. Use of pyrethroid-susceptible strains

The inclusion of a susceptible strain of mosquitoes in laboratory studies and in supplementary bioassays to semi-field studies may be an important factor to support the establishment of a baseline dataset about the product.

The susceptible strain chosen should be a laboratory strain that is 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. In a case where the fabric is intended to be effective against mosquitoes carrying a specific trait, an appropriate strain carrying the target trait should be selected as the 'baseline' strain and a scientific justification and supporting data for this selection must be provided.

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

In the early stages of the product development, the target vector population(s) for the ITN under development should be considered. This identification should include all vector populations against which the ITN is intended to be effective. For example, an ITN 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.

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

Manufacturers should consider the composition of mosquito populations, including local species/strain 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 mode of action 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, e.g. 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 two sites in geographically distinct locations should be selected for open system semi-field studies.

2.3.1. Use of mosquitoes reared from larval collections at semi-field study sites or F1 mosquitoes from study site collections

The purpose of the supplemental bioassays that are performed as part of semi-field study is to characterize the consistency of the ITNs used in the study and to provide supporting data that may assist in the interpretation of semi-field efficacy results. As such, supplementary bioassays should be conducted using either:

- adult mosquitoes reared from larval collections conducted at breeding sites located in the semi-field site *(1)*, or
- F1 mosquitoes from adult mosquito collections conducted in the study site, or
- adult mosquitoes from colonized mosquitoes originating at the study site.

In addition, manufacturers may wish to generate supplementary bioassay data using some or all the laboratory strains used to generate data during laboratory studies, or other appropriate strains if those laboratory stains are not available. The conducting of one semi-field study at the same testing facility where the laboratory study was conducted allows for coherency in datasets and aids data interpretation.

3. Characterization and monitoring of mosquito strains used in bioassays and of local vector populations at semi-field sites

Monitoring and characterization of selected strains for bioassays and wild populations should be conducted no more than 6 months before studies are initiated and no later than the end of the study in question. 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 candidate net's MOA, e.g. 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 (2)*. 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 ITN product under investigation.

4. Related documents

- WHO PQT/VCP Implementation guidance – Regeneration study for ITN fabric
- WHO PQT/VCP Implementation guidance – Wash resistance study for ITN fabric
- WHO PQT/VCP Implementation guidance – Semi-field studies for ITNs
- WHO PQT/VCP Implementation guidance – Bioassay methods for ITNs: Cone test
- WHO PQT/VCP Implementation guidance – Bioassay methods for ITNs: Tunnel test
- WHO PQT/VCP Implementation guidance – Bioassay and semi-field methods for ITNs: IACT
- WHO PQT/VCP Implementation guidance – Semi-field methods for ITNs: Experimental hut tests
- WHO PQT/VCP Implementation guidance – Matrix of selected mosquito strains
- WHO PQT/VCP Matrix of selected mosquito strains - Template

5. References

1. Guidelines for laboratory and field-testing of long-lasting insecticidal nets. Geneva: World Health Organization & WHO Pesticide Evaluation Scheme; 2013 (https://iris.who.int/handle/10665.80270).
2. 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 November 2023)