WHO Prequalification of Vector Control Products

Supporting data considerations for novel bioassays

Factors which may affect the validity of studies using novel bioassay methods:

- dossier submitted without supporting documentation to substantiate that the novel bioassay method has been validated to at least the internal validation process stage;
- novel bioassay does not demonstrate robust endpoint measurement(s) within specified precision limits;
- novel bioassay is not reproducible among multiple sites.

1. Purpose

Bioassays are used to characterise the *in vivo* effects of a substance on a test organism. For the purposes of prequalification, bioassays are laboratory or semi-field tests that use live insect vectors as the test system. Bioassays are used in data generation for Modules 3 and 5 to characterise the availability and induced effects of the formulated active ingredient(s) (AI) on mosquito test systems and on wild-free flying vector populations. The results from bioassays are used to make determinations about the consistency of the biological effect of the insecticide-treated net (ITN) fabric(s) and of entomological efficacy, and therefore, it is crucial that the bioassays selected for use are appropriate and relevant for the chemical treatments used in the ITN.

2. Bioassay methods

Historically, three bioassay methods have been used for the generation of entomological data for the prequalification of ITNs: the cone test, the tunnel test, and experimental huts. The cone and tunnel test are laboratory methods that are used to characterise the biological effect of ITN fabrics, and the experimental hut is used as an open-system semi-field method to quantify the effect of the constructed ITN on wild, free-flying mosquito vectors. More recently, the Ifakara Ambient Chamber Test (IACT) has been used as a closed-system semi-field method and as a substitute for the tunnel test to demonstrate the effect of the constructed ITN on free-flying mosquitoes.

3. Bioassays for which WHO provides implementation guidance

WHO Prequalification of Vector Control Products (PQT/VCP) does not approve bioassay methods. PQT/VCP does provide implementation guidance for the use of certain bioassay methods that have been found to generate consistent and robust data.* These methods are:

- Cone test (3-minute exposure)
- Tunnel test (15-hour exposure)
- IACT (9-hour exposure)
- Experimental huts (9-hour exposure).

The use of each method in relevant studies, e.g. the use of a cone test in a wash resistance study, is described in the implementation guidance documents for each required study. When used to generate data for Modules 3 and 5, bioassay methods must be used as described in the relevant implementation guidance documents.

*Methods to characterise the insecticide resistance status of mosquitoes, e.g. the WHO cylinder test, fall under operational guidance *(1)* that is the purview of the technical departments of WHO.

4. Use of novel bioassay methods in data generation for prequalification dossiers

Depending on the characteristics of the entomological mode of action of the AI(s) included in the formulation of the ITN under investigation, it may be appropriate to generate data using a novel bioassay method. For the purposes of prequalification, a novel bioassay is considered to be a) any method for which there is no PQT/VCP implementation guidance, or b) a method for which PQT/VCP has provided implementation guidance but which has been modified to use new experimental conditions.

In cases where a novel bioassay method has been used for data generation, method validation data for the bioassay method must be provided as part of the prequalification dossier.

4.1. Data requirements for novel bioassay methods

There are no international bodies that exist for the independent validation of bioassays. Therefore, applicants are advised to familiarize themselves with the general processes for laboratory method validation that are used in various healthcare and industry fields. In the absence of a recognized international body, for entomology method validation applicants may also refer to *A bioassay method validation framework for laboratory and semi-field tests used to evaluate vector control tools* *(2)* which suggests a framework that can be used to validate bioassay methods.

Broadly speaking, the process of method development comprises four stages:

- preliminary method development (exploratory developmental studies)
- feasibility studies (initial structured data generation)
- internal validation (full validation in one site, usually the development site)
- external validation (full validation across multiple sites to confirm method consistency).

For the purposes of prequalification, a dossier that contains data generated using a novel bioassay method should be submitted with validation data up to and including the internal validation of the method. A dossier that includes the use of a novel bioassay in multiple sites should be submitted with validation data up to and including the external validation of the method.

When the use of a novel bioassay is proposed for the evaluation of an ITN, WHO recommends that substantiating documentation be provided with a PQ200 protocol review request submission prior to the commencement of prequalification data generation.

4.2. Applicability of novel bioassays to pre- and post-market data generation

The use of novel bioassays must be consistent throughout data generation, e.g. in both laboratory studies for Module 3 and in supplementary bioassays for Module 5.

Where a novel bioassay has been identified as the sole method for the demonstration of the effects of the AI(s) on mosquito test systems, consideration should be given by the method developer to the post-market testing requirements of procurement agencies, national control programmes and member states, and ensure that post-prequalification data requirements can be met using the novel method.

When novel bioassays are implemented in additional sites beyond those used for the external validation, a method verification study must be conducted and submitted to PQT/VCP to accompany post-prequalification and post-market data submissions.

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 November 2023).
2. Matope, A., Lees, R.S., Spiers, A., Foster G. M. A bioassay method validation framework for laboratory and semi-field tests used to evaluate vector control tools. Malar J. 2023; 22:289. (https://doi.org/10.1186/s12936-023-04717-w, accessed 20 November 2023)