WHO Prequalification of Vector Control Products

Guidance for the interpretation of bioassay results without reference to pre-selected thresholds

Factors which may affect the validity of studies using product type-specific indicators:

- use of an inappropriate bioassay for the product under investigation;
- use of an inappropriate endpoint for the mode of action of the insecticide-treated nets (ITN) treatment(s);
- failure to robustly define the indicator value, including the expected precision.

1. Purpose

The prequalification assessment of ITNs has evolved from a framework for decisions that rely solely on laboratory bioassay results from tests conducted using insecticide susceptible test systems meeting preselected thresholds. This is because:

- the results from laboratory bioassays provide an indication of the bioavailability of an AI(s) on a test sample rather than a prediction of efficacy;
- the historical laboratory bioassay thresholds of ≥95% 60-minute knockdown and/or ≥80% 24-hour mortality in a cone test and ≥80% 24-hour mortality and/or ≥90% blood feeding inhibition in a tunnel test were developed for pyrethroid-treated ITNs tested against insecticide susceptible mosquito strains;
- the increase in insecticide resistance in vector populations, including plurality in both the mechanisms and intensity of resistance, requires that products are assessed against mosquito strains that carry characteristics appropriate for the demonstration of the intended effect of the product;
- products may not impact all mechanisms of resistance equally.

For the reasons above, the laboratory bioassay thresholds previously used for ITN product testing are no longer suitable for use as preselected thresholds to indicate the bioavailability of active ingredient(s) in prepared test samples.

This document provides guidance on the interpretation of bioassay results in the absence of preselected thresholds and offers an example method for setting product-specific indicator values that can be used to validate and interpret bioassay results when conducting testing using insecticide resistant test systems.

2. Identification of appropriate indicators values for use in product testing

The specific reference values, used as historical thresholds, e.g. ≥95% 60-minute knockdown and/or ≥80% 24-hour mortality in a cone test and ≥80% 24-hour mortality and/or ≥90% blood feeding inhibition in a tunnel test, may still be applicable for the interpretation of results for test products whose impact is not affected by the presence of multiple mechanisms of insecticide resistance or high intensity of resistance. In cases where these indicators have been identified as appropriate for an ITN under investigation, the applicant should:

- Provide an evidence-based scientific justification for the use of these indicators in the prequalification dossier, especially when they are used to inform further study designs. The threshold must be used consistently to interpret the results from the selected bioassay method throughout all data generation.
- Note that WHO PQT/VCP no longer accepts the use of multiple interchangeable endpoints within a single test method for product acceptability.

For products for which the historical threshold/method is unsuitable, due to either the design of the product, the characteristics of the test system and/or the intended use of the product, the applicant may determine product-specific indicator values that can be used to inform the interpretation of bioassay results.

This requires a testing facility to determine the precision with which the selected endpoint can be measured, based on the use of a strain(s) that has characteristics intended to be impacted by the ITN under investigation, and then converting the endpoint + precision measurement to an indicator value that can be used for results interpretation. The definition of product-specific indicator values should always be undertaken with support from a statistician with experience in bioassay precision analysis.

Prequalification dossiers containing the use of a product-specific threshold for a particular bioassay endpoint must contain a scientific justification for the use of the threshold including supporting data where indicated.

> The purpose of product-specific indicator values is to assign nominal values (with an appropriate measure of dispersion) that indicate the expected response for a particular test system when tested against a particular product or type of product using a particular bioassay method. These indicators can be used to validate and interpret the results generated in laboratory bioassays when conducting studies for the purposes of ITN assessments.

2.1 Example method for determining product-specific indicator values

The example below demonstrates one scenario in which it may be appropriate to determine and use a product-specific indicator**. It is important to note that this scenario is an example for the purpose of demonstrating the concept of a product-specific indicator value and that it is the responsibility of the applicant and the relevant testing facility(ies) to determine when product-specific indicator values are a) required, and b) the appropriate method and supporting documentation to submit with the prequalification dossier to support the use of the indicator value.** When the use of a product-specific indicator value is indicated, WHO recommends that substantiating documentation be provided with a PQ200 protocol review request submission prior to the commencement of prequalification data generation.

In this scenario, an ITN with a pyrethroid + PBO treatment has been developed with an intended impact on pyrethroid resistant *Anopheles spp.* vectors. The colonised mosquito strains suitable for use in bioassays at both of the proposed entomology testing facilities and in the local vector populations at the semi-field sites carry a combination of *kdr*, metabolic, and cuticular resistance mechanisms, and therefore pre-exposure to PBO is not expected to restore full susceptibility. The selected bioassay for use is the cone test and the selected endpoint is 24-hour mortality.

2.1.1. Step 1: Baseline mosquito strain data

To generate baseline data to demonstrate why using an 80% mortality threshold is not appropriate for the candidate product, the testing facilities can conduct WHO cylinder tests using pre-exposure to PBO to quantify the degree of insecticide susceptibility that is restored and conduct intensity assays to quantify the intensity of resistance. The presence of resistance mechanisms should also be characterised in the laboratory strains and in F1 mosquitoes reared from collections at the semi-field site.

Output: Baseline data set* for colonised and F1 mosquitoes

- WHO cylinder test.
- Resistance intensity.
- Resistance mechanisms.

**In a GLP testing facility that characterises its colonies and the local vector population at regular intervals, this information may be available without the need to conduct additional testing.*

2.1.2. Step 2: Baseline data from similar products

Existing data from testing facilities using the proposed mosquito strain(s), method and endpoint from tests conducted using a similar product can be collated and analysed to determine an expected endpoint measurement and precision. In this example, previous 24-hour mortality data from cone tests conducted with one or more prequalified pyrethroid + PBO products using the same or similar mosquito

strains as are proposed for testing with the product under investigation can be collated and analysed to determine the expected 24-hour mortality result within a certain precision, e.g. 65% ±5.

Output: Baseline data set* from similar products

- Expected endpoint result with precision measurement.

**In a testing facility that has not conducted previous testing using a similar product, additional tests may be required to generate this output.*

2.1.3. Step 3: Quantify product variability

Use the results from the chemical characterisation of the ITN under investigation to define the variability of the product's chemical content. This variability is used (in combination with the variability from the baseline dataset of similar products) to define the precision limit for the bioassay tests.

Output

- Defined indicator value as an expected endpoint result with a precision limit.

2.1.4. Step 4: Conduct experiments with ITN under investigation, applying the product-specific indicator value

Experiments are conducted, using the product-specific indicator value.

Output

- Experimental data set(s) characterizing the entomological effect of the fabric that are interpreted with reference to the product-specific indicator value.

3. Related documents

3.1 WHO PQT/VCP Implementation guidance – Bioassay methods for ITNs: Cone test
3.2 WHO PQT/VCP Implementation guidance – Bioassay methods for ITNs: Tunnel test