Key points and Q&A

Test procedures for insecticide resistance monitoring in malaria vector mosquitoes (Second edition, 2016)

Background

- Insecticides are critically important for malaria prevention. An estimated 663 million cases of malaria have been averted in sub-Saharan Africa since 2001 as a result of the scale-up of malaria control interventions: of these, 69% of cases were averted through the use of mosquito nets treated with insecticides and 10% through indoor residual spraying of insecticides.

- The emergence and spread of mosquitoes resistant to insecticides threaten progress made against malaria to date. To preserve the effectiveness of vector control tools, it is imperative that insecticide resistance (IR) is regularly monitored and that data generated informs programmatic action.

Rationale for guidance

- WHO launched the Global plan for insecticide resistance management in malaria vectors in 2012, which provides technical guidance for monitoring and managing the resistance status of vector populations.

- In 2013, WHO released guidelines on test procedures and operational standards for detecting the presence of IR in adult malaria mosquitoes.

- The second edition of these guidelines, released in 2016, outlines new test procedures using the current WHO tube bioassay test. These new procedures allow for the measurement of the intensity of IR and one of the main metabolic mechanisms that drives it.

- The data generated by these new test procedures will enable countries to track the evolution of IR, inform the development of national IR management strategies, and ensure the effective use of available tools.
Summary of guidance

- Insecticide resistance in mosquitoes is detected using a **susceptibility test**. This test exposes mosquitoes to an insecticide of a standard concentration for a set time period (usually one hour). Twenty-four hours after being exposed, mosquitoes are examined to determine if they are dead or alive. All susceptible mosquitoes will die, but resistant mosquitoes will remain alive.

- If fewer than 90% of mosquitoes die after insecticide exposure, it is considered confirmation that insecticide resistance is present in the local mosquito population.

- Once resistance is confirmed, further susceptibility tests with higher concentrations of insecticides should be conducted to determine if the intensity of resistance is low, moderate, or high. During an **intensity assay**, additional test mosquitoes are exposed to concentrations of the pertinent insecticides that are 5 and 10 times higher than in the first test.

- If some mosquitoes are still alive after exposure to these higher concentrations, it is an indication that the pertinent insecticide is likely to fail when used in the field. In this instance, urgent action must be taken; for example, changes in the insecticides used for indoor residual spraying.

- The involvement of metabolic mechanisms in resistance can be determined through a **synergist-insecticide assay**. This test exposes mosquitoes not only to the insecticide, but also to a chemical called a synergist that can inactivate the metabolic enzymes that can cause resistance.

- Knowledge of the metabolic mechanism underlying insecticide resistance can guide the selection of appropriate insecticidal tools that are more likely to kill mosquitoes in a target area.

Challenges

- Each test described here requires a sample of at least 150 adult female mosquitoes. Conducting an intensity assay and a synergist assay, in addition to the susceptibility test, will require at least 450 mosquitoes from a test site. Collecting enough larvae or adult mosquitoes for testing can be challenging and time-intensive. Programmes are expected to prioritize the selection of test sites based on anticipated use of data for decision-making.

Expected benefits

- These updates are intended to make it easier to generate data to guide the planning and deployment of insecticidal tools. The ultimate objective is to preserve the effectiveness of malaria vector control tools and improve management of insecticide resistance.
QUESTIONs AND ANSWERS

Interview with Dr Martha Quinones Pinzon, Technical Officer, Entomology and Vector Control, WHO Global Malaria Programme

1. What is the role of insecticides in the fight against malaria?

Insecticides are essential for effective malaria prevention. Two of our core interventions (indoor residual spraying and treated nets) rely on insecticides to kill mosquitoes when they rest on treated walls or attempt to bite humans sleeping under a treated net. Through appropriate coverage with either of these core interventions, the lifespan of the vector population decreases and malaria transmission is reduced.

2. Why has resistance to insecticides evolved?

Resistance to insecticides is generated as a result of selective pressure: insecticides kill the susceptible mosquitoes and only the resistant ones survive. Resistance is a genetic characteristic, which means these mosquitoes pass it on to their offspring. The more we use a particular insecticide on a population of mosquitoes, the more likely it is for insecticide resistance to increase in that population.

3. How concerned should the malaria community be?

We are concerned. If the insecticides we have stop working, there are few successful alternatives available. This is especially true for pyrethroids, which are currently the only class of chemicals used in bednets. Other classes of insecticides are available for indoor residual spraying, but frequent monitoring is necessary so that any signs of resistance can be detected as early as possible. The global community needs to urgently invest in the development of new insecticides with different modes of action.

4. What is being done to address the threat of insecticide resistance?

WHO launched the Global plan for insecticide resistance management in malaria vectors in 2012, which provides technical guidance for countries. Recommendations include conducting frequent monitoring of the resistance status of mosquitoes, knowing the mechanisms involved, and implementing appropriate ways to manage resistance and preserve the effectiveness of insecticides used for vector control.

5. What tests are available for assessing insecticide resistance?

WHO has a well-known test using insecticide impregnated filter papers that detects the presence of insecticide resistance phenotypes in mosquitoes. The Centers for Disease Control and Prevention (CDC) has also developed a method using insecticide-treated glass bottles. It is now possible to use either of these tests to measure the intensity of resistance and to evaluate the involvement of metabolic mechanisms in resistance. New methods using the WHO tube test are presented in the updated Test procedures for insecticide resistance guidelines that were released in November 2016. In addition, biochemical and molecular tests have been developed to detect enzymes or mutations that are conferring insecticide resistance to the mosquito populations.
6. **What was the rationale for developing new guidance on testing procedures?**

Measuring the intensity of resistance and the underlying metabolic mechanisms will help programme managers make decisions on how best to manage insecticide resistance. Areas with moderate or high intensity of resistance can then be prioritized for appropriate adjustments in interventions – for example, changes in insecticides. Knowledge of the metabolic mechanism helps inform the best approach for preventing resistance to multiple insecticides.

7. **Please describe what is new in the most recent guidance.**

This second edition is an update of WHO’s 2013 guidelines and addresses ways to measure not only the presence of insecticide resistance in malaria vectors, but also its strength through the use of resistance intensity assays. This will allow for the identification of regions and areas where resistance is most intensively expressed and, therefore, areas where insecticide-based vector control interventions are most likely to fail. Also, a new method was included to determine the metabolic mechanism underlying insecticide resistance, which can help determine which insecticides will be affected by the resistant mosquito population.

**ONLINE RESOURCES**


http://who.int/malaria/publications/atoz/9789241511575/en/