Bioassay Monitoring methods

WHO test kit: for adults and larvae
Adults: The kit and papers can be purchased with full instructions on their use. Supplier details can be found at: www.who.int/whopes/resistance/en/


CDC bottle test kit (+/- synergists)

Synergists, such as PBO and DEF, can be used with the insecticide in the bottle bioassay and can give a limited indication of metabolic resistance mechanisms, if present.

Biochemical Monitoring methods

These methods rely upon enzymatic action upon a model substrate which may, or may not, accurately reflect metabolism of insecticidal compounds. Typically P450 activity is measured using O-deethylation of 7-ethoxycoumarin, glutathione-S-transferase activity is measured using chlorodinitrobenzene (CDNB) or dichloronitrobenzene (DCNB) and non-specific esterase activity using 1-naphthyl acetate. Using microplate technology these spectrophotometric/ fluorometric assays can become powerful, high-throughput monitoring assays.

Molecular Monitoring methods

Molecular assays can greatly complement bioassays. Their use is currently restricted to research labs since field test kits are still in development. The following is a description of some useful molecular techniques:

- Species identification using a multiplex PCR assay. The proportion of each species that were survivors and non-survivors of bioassay susceptibility tests can be determined.
- Identification of M and S molecular form within Anopheles gambiae s.s. can be differentiated using a restriction fragment length polymorphic (RFLP) PCR assay. The proportion of each molecular form that were survivors and non-survivors of bioassay susceptibility tests can then be determined.
- Detection of kdr by Polymerase Chain Reaction allele specific RT-PCR (Reverse Transcription Polymerase Chain Reaction) assays can be carried out on phenotyped samples using a restriction fragment length polymorphic (RFLP) PCR assay. The frequency of kdr alleles can also be determined within each of the M and S molecular forms of An. gambiae s.s. and within survivors and non-survivors of bioassay susceptibility tests.
- Microarray: to screen for metabolic resistance from field caught mosquitoes (e.g. An. gambiae s.s., An. arabiensis, An. funestus and Aedes aegypti). The ‘detox chips’, which were developed at the Liverpool School of Tropical Medicine, can be used to identify RNA levels associated with P450 oxidase, GST and esterase based resistance.

The importance of biomolecular techniques

- There are some challenges associated with bioassays that may make data interpretation more complicated. For example; when field collected mosquitoes are used, often not enough adults can be found and those found will be of mixed age and blood fed status and may have had prior exposure to insecticides. If Larvae are collected F1 adults are used, access to a lab/insectary is required and results with F1 may not be fully representative of the local population.
- Biomolecular techniques can identify heterozygous resistant individuals which are not easily identified in bioassays and can identify resistance mechanism/s in an individual mosquito or a population.
- A clear understanding of the resistance mechanisms present in a population is important for choosing the most effective vector control tools, which should be an integral part of insecticide management strategies.

Further information in Chapter 7: Prevention and management of insecticide resistance in vectors and pests of public health importance, www.irac-online.org