The increase in the utility and importance of biomolecular techniques in resistance monitoring in insect vectors

Insecticide Resistance Action Committee

www.irac-online.org

Monitoring Objectives

The monitoring of insecticide susceptibility in vector control programmes has three main activities:

• **Baseline data collection**: Conducted prior to the start of a control programme in order to provide baseline data to inform planning and insecticide choice.

• **Monitoring of susceptibility over time**: To evaluate the proportion of susceptible mosquitoes in the population over time, comparing it with the pre-intervention baseline. Hence the impact of the control strategy on the proportion of susceptible individuals in the mosquito population can be evaluated.

• **Detection of resistance**: To detect resistant individuals when they are at a low frequency in the population so that resistance management can be effectively introduced. Detection of resistance when a large proportion of the mosquito population are already resistant limits the potential effectiveness of IRM strategies.

Careful use of the information generated from the activities above will allow evidence based decisions to be made in the design of integrated vector management strategies in a specific locality

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/

Larvae: Details for the test method may be found at: www.who.int/whopes/guidelines/en/ - Guidelines for laboratory and field testing of mosquito larvicides. (WHO/CDS/WHOPES/GCDPP/2005.13).

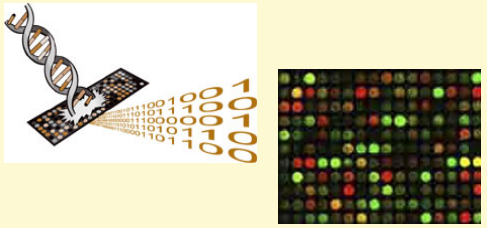
CDC bottle test kit (+/- synergists)

Full details and a step-by-step method description are available from www.cdc.gov/ncidod/wbt/resistance/assay/bottle/index.htm

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.



Diagrammatic representation of microarray 'Detox' Chip and photographic output

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 or 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

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