TEST PROCEDURES FOR INSECTICIDE RESISTANCE – TECHNICAL UPDATE 2016

ENTOMOLOGY AND VECTOR CONTROL UNIT

Global Malaria Programme World Health Organization
Insecticides are an important element of an integrated approach to vector control

Predicted cumulative number of malaria cases averted by interventions in sub-Saharan Africa (2000-2015)

- Cases averted due to ITNs
- Cases averted due to ACTs
- Cases averted due to IRS
- Total averted not attributable to IRS, ITNs, or ACTs

Source: Malaria Atlas Project (18) estimates of cases averted attributable to ITNs, ACTs, and IRS and WHO estimates of total cases averted

World Malaria Report 2015
[Definition of insecticide resistance: The property of mosquitoes to survive exposure to a standard dose of insecticide; may be the result of physiological or behavioural adaptation. Note: The emergence of insecticide resistance in a vector population is an evolutionary phenomenon due to either behavioural avoidance (e.g. exophily instead of endophily) or physiological factors whereby the insecticide is metabolized, not potentiated, or absorbed less than by susceptible mosquitoes.]
Resistance to four classes of recommended insecticides for adult malaria vector control has been reported for all WHO regions.
Insecticide resistance (IR) surveillance – part of entomological surveillance

- Sentinel sites for IR surveillance
- Mechanisms of IR

Strategies for resistance management (GPIRM):

*Management of resistance strategies before resistance is found*

Framework for action in key areas:

- Planning and implementing national IR management strategies.
- Ensuring entomological and susceptibility monitoring and effective data management.
- Developing new, innovative vector-control tools.
- Filling knowledge gaps on resistance mechanisms and the impact of current insecticide resistance management approaches.
- And enabling mechanisms to improve advocacy and build human and financial resources.
EVALUATION OF FIELD OPERATIONS IS ALSO CRITICAL

✓ Quality of operations
✓ Quality of insecticides
✓ Efficacy on the insect populations and disease
Current guidelines use Discriminating Dose Assays

- Discriminating dose bioassays detect the presence of resistant individuals in the population.

- They do not measure the level of resistance, nor do they give any information on the likely impact of resistance on control endpoints.

- They cannot be used to compare the level of resistance between insecticides or between populations.
### Cross resistance patterns of different classes of insecticide

<table>
<thead>
<tr>
<th>Biochemical mechanism of resistance</th>
<th>Metabolic</th>
<th>Target-site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Esterases</td>
<td>Mono-oxygenases</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Pyrethroids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbamates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organophosphates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From reference (27)
GSH, glutathione; AChE, acetylcholinesterase; circle size reflects relative impact of mechanism of resistance

*GPIRM Global Plan for Insecticide Resistance Management in malaria vectors. WHO, 2012*
In order to guide potential deployment of PBO LLINs, countries considering pilot exploratory implementation should be supported to:

- collect data on the presence, level, **intensity and mechanisms of resistance** to all insecticide classes at representative sentinel sites;
- design an evaluation with appropriate indicators based on detailed guidance.
Test procedures for insecticide resistance monitoring in malaria vector mosquitoes
Second edition

Photo: GMP, 2016
MONITORING INSECTICIDE RESISTANCE

WHO susceptibility test

CDC bottle bioassay

Photo: GMP, 2016
Updates to the 2013 guidelines are needed to include:

- **Intensity bioassay**: measures the strength of resistance in a population

- **Synergist bioassay**: indication of involvement of metabolic mechanisms in resistance
NEW METHODS

INTENSITY ASSAY

• Conducted once resistance is confirmed using the discriminating concentration

• Involves exposing additional test mosquitoes from the same site to 5X and 10X the discriminating concentration

• Observed resistance is then classified as:
  • Low intensity resistance
  • Moderate intensity resistance
  • High intensity resistance

• Information is used to:
  • monitor the evolution of IR
  • make decisions
## DISCRIMINATING AND INTENSITY CONCENTRATIONS

<table>
<thead>
<tr>
<th>Insecticide class</th>
<th>Insecticide</th>
<th>Discriminating concentration (%) (1-hour exposure period)</th>
<th>5×&lt;sup&gt;a&lt;/sup&gt; concentration (%) (1-hour exposure)</th>
<th>10×&lt;sup&gt;a&lt;/sup&gt; concentration (%) (1-hour exposure)</th>
<th>Control paper</th>
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</thead>
<tbody>
<tr>
<td>Carbamates</td>
<td>Bendiocarb</td>
<td>0.1</td>
<td>0.5</td>
<td>1</td>
<td>Olive oil</td>
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<td>Carbosulfan</td>
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<td></td>
<td></td>
<td>Olive oil</td>
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<tr>
<td></td>
<td>Propoxur</td>
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<td></td>
<td></td>
<td>Olive oil</td>
</tr>
<tr>
<td>Organochlorines</td>
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<td>4</td>
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<td></td>
<td>Risella oil</td>
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<td>Dieldrin</td>
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<td></td>
<td></td>
<td>Risella oil</td>
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<td>4</td>
<td></td>
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<td>Risella oil</td>
</tr>
<tr>
<td>Organophosphates</td>
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<td></td>
<td></td>
<td>Olive oil</td>
</tr>
<tr>
<td></td>
<td>Malathion</td>
<td>5</td>
<td></td>
<td></td>
<td>Olive oil</td>
</tr>
<tr>
<td></td>
<td>Pirimiphos-methyl</td>
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<td>1.25</td>
<td>2.5</td>
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<tr>
<td>Pyrethroids</td>
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<td>0.25</td>
<td>0.5</td>
<td>Silicone oil</td>
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<td>Cyfluthrin</td>
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<td>1.5</td>
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<td>Deltamethrin</td>
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<td>0.25</td>
<td>0.5</td>
<td>Silicone oil</td>
</tr>
<tr>
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<td>Etofenprox</td>
<td>0.5</td>
<td>2.5</td>
<td>5</td>
<td>Silicone oil</td>
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<td>Lambda-cyhalothrin</td>
<td>0.05</td>
<td>0.25</td>
<td>0.5</td>
<td>Silicone oil</td>
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<td>Permethrin</td>
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<td>3.75</td>
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<td>Phenylpyrazoles</td>
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<td></td>
<td></td>
<td>Silicone oil</td>
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<tr>
<td>Synergist</td>
<td>Piperonyl butoxide</td>
<td>4</td>
<td></td>
<td></td>
<td>Silicone oil</td>
</tr>
</tbody>
</table>

Test Procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2016

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*a* Concentrations are expressed in terms of the pure pesticidal compound only, and not the formulation or solvent used.

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**Remarks**

Carbamates

- Bendiocarb: Resistance is common.
- Carbosulfan: Resistance is common.
- Propoxur: Resistance is common.

**Organochlorines**

- DDT: Resistance is common.
- Dieldrin: Resistance is common.

**Organophosphates**

- Fenitrothion: Resistance is common.
- Malathion: Resistance is common.
- Pirimiphos-methyl: Resistance is common.

**Pyrethroids**

- Alpha-cypermethrin: Resistance is common.
- Cyfluthrin: Resistance is common.
- Deltamethrin: Resistance is common.
- Etofenprox: Resistance is common.
- Lambda-cyhalothrin: Resistance is common.
- Permethrin: Resistance is common.

**Phenylpyrazoles**

- Fipronil: Resistance is common.

**Synergists**

- Piperonyl butoxide: Resistance is common.

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Based on data published by N’Guessan et al. (2003) and Ahoua Alou et al. (2010).
**INTERPRETATION OF INTENSITY ASSAY RESULTS**

- **Discriminating conc. (1X)** < 98% mortality
  - Intensity conc. (5X) ≥ 98% mortality
    - (no need to expose to 10X)
  - **LOW INTENSITY RESISTANCE**

- **Discriminating conc. (1X)** < 98% mortality
  - Intensity conc. (5X) < 98% mortality
    - Intensity conc. (10X) ≥ 98% mortality
  - **MODERATE INTENSITY RESISTANCE**

- **Discriminating conc. (1X)** < 98% mortality
  - Intensity conc. (5X) < 98% mortality
    - Intensity conc. (10X) < 98% mortality
  - **HIGH INTENSITY RESISTANCE**
SYNERGIST ASSAY

- Pre-exposure to synergists

- Synergist inhibits enzymes. For example, PBO inhibits oxidases.

- Because enzyme is inhibited, insecticide reaches target and mosquito dies (i.e. susceptibility is restored)

Test Procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2016
INTERPRETATION OF THE SYNERGIST BIOASSAY RESULTS

**Insecticide exposure**

- Mean mortality < 90%
- Effect of synergist (PBO) can not be reliably assessed

**Pre-exposure to synergist (PBO) then insecticide exposure**

- Complete restoration of susceptibility
  - monooxgenase-based resistance mechanism **fully accounts** for expression of the resistant phenotype
- Partial restoration of susceptibility
  - monooxgenase-based resistance mechanism **only partially accounts** for expression of the resistant phenotype
- No restoration of susceptibility
  - the resistance phenotype detected is **not based on** monooxgenase-mediated detoxification
INTERPRETATION OF THE SYNERGIST BIOASSAY RESULTS

Possible outcomes of synergist bioassay tests

- Mortality rate %
- TOTAL RESTORATION
- PARTIAL RESTORATION
- NO RESTORATION

Insecticide alone
Insecticide + PBO
Process for insecticide susceptibility testing

Susceptibility test with discriminating concentration (1×) (see Section 3.1)
- ≥ 98% mortality: Susceptible
- 90–97% mortality: Possible resistance
- < 90% mortality: Confirmed resistance

Repeat test (if)<br>
< 98% mortality: Confirmed resistance

To determine resistance intensity

Susceptibility test with discriminating concentration (5×) (see Section 3.2)
- ≥ 98% mortality: Low intensity resistance
- < 98% mortality: Moderate to high intensity resistance

Susceptibility test with discriminating concentration (10×) (see Section 3.2)
- ≥ 98% mortality: Moderate intensity resistance
- < 98% mortality: High intensity resistance

To determine resistance mechanism(s)

Synergist-insecticide bioassay (see Section 4)

Molecular or biochemical assays (see Section 6)

See Section 4.3 for interpretation of test results

Outcome and interpretation depends on test used:
- a. WHO insecticide susceptibility test or US Centers for Disease Control and Prevention (CDC) bottle bioassay following standard procedures and using defined dose/concentration with adjustment of mortality outcomes if necessary.
- b. Conducted using untested mosquitoes from the same population.
- c. Can be conducted using progeny of surviving mosquitoes from bioassays (F1 reared under laboratory conditions).
- d. Can be conducted using mosquitoes tested in bioassays.
- e. Test for known resistance mechanisms only.

Test Procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2016
Figure 30: Three factors to be assessed when a new focus of insecticide resistance is identified

Link to the *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Second Edition*: 
http://www.who.int/malaria/en/ (Latest guidance section)

http://apps.who.int/iris/bitstream/10665/250677/1/9789241511575-eng.pdf?ua=1

QUESTIONS?

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