Insert 2 - Enhanced Surveillance Protocol for the CDC Intensity Bottle Bioassay - 2013

Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay

Background

Up to now, the major methods of anopheline bioassay, the WHO tube assay and the CDC bottle assay, have focused on determining the frequency of insecticide resistance relative to a discriminating (also referred to as diagnostic) dose of insecticide for a pre-determined diagnostic period of time. Although these methods generally agree on resistance frequencies, it has become apparent, through our extensive experiences in Africa, Asia and the Americas as part of the President's and Amazon Malaria Initiatives, that resistance frequency data at best provide only weak evidence to support the crucial decisions that must be made in procurement and deployment strategy for public health pesticides.

We are learning that the practical information of greatest significance in decision-making involves resistance intensity. For example, let us suppose that two populations of anophelines (in different areas within a country) show a resistance frequency of 25% in any of the accepted bioassay formats. If none of the resistant mosquitoes in site A can survive twice the diagnostic dose of a particular insecticide and those at site B have 15% survive 5 or even 10 times the diagnostic dose at the diagnostic time, the decisions going forward for those locations would need to be completely different. We are accumulating field evidence that such situations are occurring regularly within the area of country malaria control programs, for example in Zambia, Mali and Ethiopia.

Collection of Mosquitoes

The method of collecting mosquitoes is crucial to this protocol, since it is designed as a rapid diagnostic test (RDT) for resistance and its mechanism(s). There is no ideal method of collection, but for an RDT we must have field-collected adult mosquitoes. For indoor feeding mosquitoes the most efficient method of collection is use of a backpack aspirator early in the morning (4 AM-dawn). Care must be taken to change the collecting cup at no more than 5-minute intervals to reduce stress on the collected insects. Prior to assay, a holding period of two hours is advisable to allow any damaged individuals to fall out. Hand aspiration can be useful if mosquito populations are very high, but the number of mosquitoes collected and the speed with which 10-20 houses may be sampled with the backpack aspirator makes it the method of choice.

There are several disadvantages to collecting adult females for rearing of F1s. First, the benefits of an RDT are lost due to the time and facilities needed for mosquito rearing. Secondly, a large number of females must lay eggs and be sampled to allow any hope of comparison of resistance frequency to the field population. Also, resistance intensity is difficult to assess because of the variability of rearing conditions of the F1s and the absence of the selection history characteristic of the infected older mosquitoes. Finally, the mosquitoes assayed do not include the age distribution of infected mosquitoes or of those potentially acquiring infection at the time of collection. The problems with larval collection

are greater, since both indoor-feeding and early feeding-outdoor feeding sibling species mosquitoes are collected indiscriminately from breeding sites and must be identified using molecular techniques for data interpretation. The other problems associated with reared mosquitoes are as seen with F1s.

This is not to say that larval collections and rearing of F1s should not be used for the intensity assay. In many programs the practicality of collections may outweigh the advantages of the collection method described above. The important thing is that resistance surveillance be a high priority of any malaria vector control program.

Resistance Frequency Rapid Diagnostic Test (F-RDT)

The resistance frequency RDT is simply the existing bottle bioassay protocol conducted upon field-collected adult female mosquitoes. The assays are run upon the mosquitoes available. Up to 25 mosquitoes per assay replicate (up to four replicates and a control) are exposed to the diagnostic insecticide dose for the diagnostic time. It is best to divide the available mosquitoes across four replicates (assuming there are enough). Data may be accumulated (pooled) from a specific site (e.g. village), but resolution of resistance foci may be lost if data are pooled over a wider area.

Resistance Intensity Rapid Diagnostic Test (I-RDT)

The simplest resistance intensity RDT uses bottles (one per dose) treated with 1, 2, 5 and 10 times the diagnostic dose of insecticide plus a control. The diagnostic time is not altered. If only 20 mosquitoes are collected, four mosquitoes would be introduced into each bottle. Obviously, more mosquitoes would be needed for a confident assessment of resistance intensity at a particular site, but note the value of knowing if even one or two mosquitoes can survive at the 5x and 10x dosages. That would serve as an early warning that a particular site needs much closer surveillance. Given a large number of available mosquitoes, up to 25 insects per bottle give more reliable information on the intensity population structure and, most ideally, four replicates of each dosage can be run. Note that higher dosages than 10x may be needed to establish maximum intensity levels in some mosquito populations. In Zambia, we observed over 30% survival at 10X in some *Anopheles funestus* populations. It is likely that 20, 50, or 100x may have relevance at present or eventually in Africa. Note also, that comparison of resistance intensities among pyrethroids will directly measure the potential for differential toxicity to allow more creative uses of pyrethroid subclasses and individual insecticides in combinations or rotations for resistance management.

Resistance Mechanism Rapid Diagnostic Test (M-RDT)

Once resistance frequency or intensity are determined, discrimination between metabolic and target site resistance may be estimated using synergists. Although the specificity of some synergists for particular metabolic mechanisms can be ambiguous (e.g. piperonyl butoxide (PBO) for oxidases and esterases in longer protocols than we use), the particular problem in Africa of joint action of kdr and

oxidases is accessible to this technique. In the assay, the collected mosquitoes are divided between those exposed for one hour in a control bottle and those exposed to the diagnostic dose of a synergist. Following this pre-exposure, the two experimental populations of mosquitoes are subjected to the I-RDT test. The effect upon resistance frequency (the 1x bottle) and intensity (2, 5 and 10X bottles) can be directly measured. Note also, that the potential differences between pyrethoids and/or their subclasses based on oxidase specificity may be directly assessed using this protocol.

Intended Impact

This protocol is designed to allow assessment of the maximum number of sites at least cost throughout program decision-making to be based upon a more complete understanding of resistance significance at varying frequencies and intensities and to also allow assessment of the strength and resistance mechanism profile of resistance foci. Resistance management strategies based upon such information may be expected to be significantly less expensive to implement.