



PRESIDENT'S MALARIA INITIATIVE



GUINEA: Training in Basic Malaria Entomology and Vector Control August 2012

Integrated Vector Management (IVM) Task Order 2

Contract GHA-I-02-04-00007-00

Prepared for:
United States Agency for International Development

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September 2012

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Consultant Report¹

Training in Basic Malaria Entomology and Vector Control Maferinya, Guinea 6-16 August, 2012

**By
Dr Martin Akogbeto**

¹ The report has been edited by the IVM Project.

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1.0 INTRODUCTION

PMI is supporting the Government of the Republic of Guinea in malaria control. As part of ongoing efforts, the IVM project was tasked to support country capacity for entomological surveillance and monitoring. A 2-week training in standardized methods in entomology surveillance and vector control was organized 6 – 16 August, 2012 at the Center for Research, Maferinyah, Guinea. The goal of the training was to create a critical mass of technicians who will initiate baseline entomological monitoring of malaria vectors.

Professor Martin Akogbeto, a noted African entomologist, was contracted by the IVM project to conduct the training. Prof. Akogbeto had previously supported similar training in DRC and Burundi for the IVM project.

1.1 Opening Session

The official start of the training took place on Monday, August 6, 2012 at 9 o'clock at the Rural Health Training and Research Center, Maferinyah, with a welcome statement by Dr Beavougui, Director of the Center. Dr Ibrahim Kalil Kéita, Head of the Vector Control Unit of the National Malaria Control Program (NMCP) and representative of the Director of the Program, thanked the United States Agency for International Development (USAID), RTI International, 'Faisons Ensemble' Project and the Rural Health Training and Research Center of Maferinyah, for initiating and funding the training. He stressed that entomological research is the weakest aspect of malaria research in Guinea. Consequently, there is very little data available on malaria entomology in Guinea. Dr Ibrahim Kalil Kéita, who doubled as the representative of the Director of the NMCP, launched the training applauding the opportunity to provide Guinea health agents a training on basic entomology to help the National Malaria Control Program to more effectively monitor the dynamics of malaria vectors and their susceptibility to WHOPEs approved insecticides.

2.0 TRAINING

A pre-test aimed was conducted at the start of the training to assess the level of each trainee in basic malaria entomology, since they did not all enter the training with the same level of background knowledge on entomology.

The training was divided into two main sections: a theoretical section, and a practical section which took place both indoors and out in the field. The points of the training covered the following topics:

- Identification of malaria vectors (mosquito morphological identification),
- Techniques for sampling larvae and adult mosquitoes,
- Mosquito breeding in the laboratory/insectary,
- Insecticide susceptibility testing and interpretation of results,
- Cone bioassays and interpretation of results,
- Malaria stratification,
- Incrimination of malaria vectors (density calculation from two types of sampling: night human landing catches inside and outside houses and pyrethrum spray catches, etc.),
- Malaria vector control,
- Transport and conservation of mosquito samples

The training curriculum can be found in Appendix 2.

2.1 Theoretical Sessions

The theoretical sessions began on Monday, August 6, with an overview presentation on the ten-day training, including a review of the context and expectations of the training. The presentation was followed by an introduction to malaria entomology. The unit focused on the description of the malaria transmission cycle of malaria, the description of the mosquito's life cycle in relation to malaria transmission, as well as the purpose and the role of entomological evaluations in malaria control.

The theoretical sessions were mainly based on PowerPoint presentations, allowing adequate time for participants to ask questions and seek clarification. Demonstrations were performed after some of the theoretical sessions to help the trainees better-understand the material.

The WHO document entitled 'Malaria entomology and vector control' (WHO/CDS/CPE/2002. 18 Rev. 1, Part 1, French version, Temporary Edition, July 2003) was printed as a booklet and distributed to the trainees to use as reference source.

At the beginning of each day's session, a report on the activities of the previous day was presented by two nominated participant-reporters and validated by their fellow participants through plenary discussions. This was an easy exercise, as the trainees already had a good level of understanding of the material; a number of physicians and laboratory managers took part in the training.

2.2 Practical Sessions

The following basic techniques were reviewed:

2.2.1 Morphological identification of mosquitoes

The trainees learned how to morphologically distinguish between *Anopheles* and the other Culcidae using the naked eye and under the stereomicroscope. The recognition of mosquitoes at the sub-families level (Anopheline: *Anopheles*, Culiciné: *Culex*) seems well-understood. This was very difficult for some of the trainees at first, and a number of practice sessions as well as experiences from the field trips described below, were necessary to ensure they all fully understood the technique. At the end of the 2-week training, all trainees has fully mastered the identification of the 2 main types of mosquitoes.

2.2.2 Mosquito larvae sampling techniques

The first two Field trips occurred following the theoretical sessions on Monday, August 6 and Tuesday, August 7. The field practical sessions focused on identifying mosquito larval breeding sites, collecting and identifying larvae of *Anopheles gambiae* and *Culex*, sorting the larvae and the pupae, and breeding mosquitoes. Two larval surveys were conducted on in a rice growing area not far from the National Rural Health Training and Research Center of Maferinyah (NRHTRCM). Although the majority of water points encountered were ideal breeding sites, they were mostly negative for larvae and pupae at that time of year. Hence, very few larvae of *Anopheles gambiae* and *Culex* were collected. The collected larvae were returned to the laboratory at NRHTRCM for further study. The *Anopheles* larvae were separated from those of *Culex*. The larval instars were sorted and separated. There were two larval trays: one for the *Anopheles* larvae and one for the *Culex* larvae. The trainees were taught how to feed the larvae contained in the trays with appropriate quantity of fish feed to avoid an overload in the trays. There used pipettes to sort out the nymphs.

Every day, the trainees cared for the mosquito colonies (sorting the pupae, feeding the larvae and the adults). The adults emerging from the larval colony in the laboratory were kept in cages at the lab and instructions provided to ensure that the cages were protected from ants. The trainees also got conversant with the room temperature and humidity requirement for rearing mosquitoes in the lab. The emerging adults were subsequently used for training in susceptibility tests utilizing WHO tubes, as well as for the cone bioassays.

2.2.3 Adult mosquito sampling techniques

Field trip 3, focused on the collection of adult mosquitoes after indoor residual spraying of insecticide, sorting and classification of collected mosquitoes, according to the species type and also by the state of the abdomen (unfed, fed, semi-gravid, and gravid).

On Thursday, August 9, the trainees conducted pyrethrum spray catches (PSC) early in the morning in an area close to the training center. A total of 9 huts (rooms) were used. During the PSC, the number of people who slept the night before, as well as the presence or absence of mosquito nets, were noted. The mosquitoes collected were identified, some done on site and the others back at NRHTRCM. The Anophelines were separated from the Culicines. *An. gambiae* were identified, after which males were separated from females. Mosquitoes were separated into four groups: unfed, the fed, semi-gravid and gravid. The mosquito density per hut and the biting rate (number of bites per man per night) were estimated. The average density of people per hut and the average number of nets per household were calculated (6 nets for 37 people). Only five *Anopheles* (all of them being *Anopheles gambiae*) out of the 258 mosquitoes collected after PSC were identified. Despite the presence of nets in most of the houses, the majority of the mosquitoes collected were found with relatively fresh blood in the abdomen except in one of them (a household with four people and four nets) where the majority of the mosquitoes were unfed. The Class then used the number of fed and semi-gravid mosquitoes, as well as the number of people who slept in the room the night before, to estimate the biting rate, with the relevant assumptions that (i) all freshly fed mosquitos obtained blood from the occupants of the room that night and (ii) that no freshly fed mosquito exiting the house the night before the PSC.

Field trip 4 (Night landing catches): This took place from 08:30 p.m to 10:00 p.m., on Friday, August 10 2012. The practical provided trainees with in-depth experience on the methodology for human night landing catches. It took place within the grounds of the NRHTRCM, with the trainees as mosquito

collectors. A large number of mosquitoes were captured by all trainees (unfortunately, there were all *Culex*). The mosquitoes caught during the HLC, were used for subsequent studies during the training course.

Field trip 5 (Resting collection of adult mosquitoes): On Saturday, August 11, resting collection of adult mosquitoes was conducted in an area close to the NRHTRCM. The trainees collected all mosquitoes found resting in the houses in the areas. Most of the mosquitoes collected were *Culex*. The only *An. gambiae* found during the collection was subsequently used as part of sample for training on susceptibility and cone bioassay tests –complementing the adults emerged from the laboratory reared larval colony from field trips 1 and 2. The scarcity of *An. gambiae* during that training period was probably due to the high frequency of heavy rains during the period; It rained non-stop every day for most of the training course period, and the rain water overrun and washed away the breeding sites and *An. gambiae* larvae. The unfortunate occurrence, however provided a critical lesson in entomological monitoring for the trainees on breeding ecology and factors that impact successful breeding of *An. gambiae*.

2.2.4 Vector insecticide susceptibility testing and interpretation of results

A demonstration of the susceptibility tests was performed by the consultant before the trainees performed the tests. During the tests, conditions required for testing (especially temperature and humidity conditions) and what to avoid during manipulations in order not to traumatize the mosquitoes were emphasized. In the absence of a sufficient number of mosquitoes due to the season, the trainees performed the insecticide testing with the *Culex* females that they captured. They used paper impregnated with permethrin 0.75% for the testing. Then they learned how to record the results on a sheet that was given to them and how to interpret the data (fully susceptible or resistant population and suspecting resistance according the WHO criteria).

2.2.5 Cone bioassay

An LLIN was used for the tests. The correct and proper procedure for the cone bioassay was emphasized. Following prior discussions between the IVM project and the trainer-consultant, the latter brought along some WHO kits, which enabled the cone bioassay training to take place, as those ordered by the IVM project had not a yet arrived from Malaysia and there were none available in country.

2.2.6 Labeling and preservation of mosquito samples

This section was considered very important because one of the roles of the new technicians in medical entomology will be to transport samples collected at the provinces to the research centers for further evaluations. Aspects emphasized included proper labeling, prevention of sample mix-ups, proper preservation, and avoiding risks to sample integrity during transportation.

2.2.7 Breeding of mosquitoes in the laboratory

Although, the insectary of the entomology laboratory of the NRHTRCM was not yet functional at the time of the training, it was possible to breed the mosquitoes in one of the Parasitology labs of the center. The training emphasized the basics of proper mosquito breeding, conditions and techniques, including temperature and humidity regulation, feeding of larvae and adults, sorting of larvae and pupae, preventing escape of adults, preventing contamination of colonies etc.

3.0 LOCAL PROCUREMENT OF SUPPLIES

Taking a cue from the various experiences had in previous trainings in the Democratic Republic of Congo and in Burundi, The IVM Project, in close coordination with the leadership of the ‘Faison Ensemble’ Project, NMCP and the Consultant, arranged for local procurement of minor equipment and supplies. This mainly included equipment for collecting and carrying mosquito larvae in the field, breeding and studying mosquitoes etc.

4.0 CONCLUSION

The training was a success, and the goals are reached. The trainees received a good basic training and are now equipped to identify the breeding sites of *Anopheles* larvae in the field, collect the larvae, breed them to get adults and perform tests of susceptibility to insecticides, perform sampling of the adults using human landing catches and after PSC, identify mosquitoes at the genus level and transport them under good conditions. At the end of the training, fruitful discussions led to recommendations in basic entomology field.

5.0 ACKNOWLEDGMENT

I would like to thank the NMCP/Guinea, the USAID/PMI and RTI International, for the opportunity to lead the training. I would also like to thank the ‘Faisons Ensemble’ Project under the leadership of Dan

Gerber, the National Rural Health Training and Research Center of Maferinyah and its Director for hosting the training and hospitality the moderators, as well as the reporters who helped in managing the plenary debates/discussions and recording the content of the training. I would also like to congratulate Dr. Kalil, entomologist at the NMCP, for his good organization of the training. Finally, I wish to thank the trainees with whom I worked in conviviality. They were very enthusiastic learners, hardworking and very disciplined -always on time for the courses.

ANNEX 1: SCOPE OF WORK FOR CONSULTANCY

Dr Martin Akogbeto (6 - 16 August 2012)

Background

The U.S. Agency for International Development (USAID) offers technical and financial support at the global and country levels for the implementation of malaria and other vector borne disease control activities. Under the Integrated Vector Management Task Order II (IVM 2), the Research Triangle Institute (RTI International) is providing technical assistance resources to institutionalize best practices, conduct operational research, and strengthen the management capacities of country programs. The objective is to advance the state of the art of vector control to facilitate and sustain the effective management of disease vectors and reduce local disease burdens. IVM 2 compliments the overall strategy of the President's Malaria Initiative (PMI) in Africa.

As part of the above mandate, the project is supporting the development national capacities in USAID focus countries for entomological monitoring and surveillance to support vector control implementation. Activities include conducting targeted training and establishing insectaries and entomology laboratories and development of national vector surveillance schemes. As part of the 2012 PMI support to Guinea, the IVM project will organized a 2-week basic entomology training course from 6 – 16 August 2012 for selected technicians of the national malaria control program (Programme National Integre De Lutte Contre Le Paludism - PNLN). This will include the entomologists of the MOH (consisting of entomologists located at the PNLN, t the National Public Health Laboratory and the center for research in Maferinyah), as well as entomology focal points selected from within the 4 ecological zones. The objective of the training will be to create a critical mass of trained technicians to support routine entomological monitoring activities linked to the vector control operations. The training will cover the following areas:

- Basic malaria eco-epidemiology- including role of vector(s), primary interventions
- Species identification (morphological)
- Adult and larval sampling techniques
- Transmission indices (pyrethrum catches for density assessments, landing catches -outdoor/indoors etc.)
- Fundamentals of laboratory rearing of mosquitoes
- Wall bio-assays on insecticide decay rates and knock down assessments on LLINs
- Vector susceptibility evaluations
- Preparations, labeling and storage of vector samples
- Basics of insectary management

Purpose of STTA

Dr Martin Akogbeto will be engaged as consultant 6 – 16 August 2012, to support the proposed basic entomology technician training course in Guinea. The Consultant will work closely with the PNLN and the IVM Project Staff. Dr Akogbeto to undertake the following tasks:

- Support the finalization of curriculum and technical content of the training;
- Serve as the expert instructor during the training;
- Provide advisory functions to guide the NMCP as it develops operational plans for entomological monitoring;

Deliverables

- Administer 2-week training of about 26 entomology technicians in Guinea in August 2012 and provide a mission report that provides details of the training course.
- Report of the training activity by August 29, 2012.

Level of Effort

15 full-time work days at 6 days a week.

ANNEX 2: CURRICULUM-ENTOMOLOGY TECHNICIANS TRAINING (BASIC LEVEL)

Purpose of training: The training is aimed at supporting efforts by the national malaria control program (NMCP) to build a critical mass of trained staff at the central and district levels, for entomology surveillance and monitoring to guide malaria vector control interventions. The training will provide entomology technicians with basic knowledge on the role of vector control in malaria control, the biology and control of mosquito vectors, as well as competency in standardized methodologies for the surveillance and monitoring of malaria vectors. The training will focus on hands-on practical experience and ensure that vector monitoring activities in Guinea are uniform and follow the same strict procedures to enhance reliability of results for decision making.

All the areas to be covered by the training are outlined in-depth in the *Manual for Entomology Technicians training* (basic level), drafted by the IVM Project. The draft manual will serve as the basic document guiding the training. The purpose, content of each subject area and associated field activities are as follows.

Topic	Purpose	Associated Activities
Malaria vector control (basic principles)	Vector control is a major element of the Global Malaria Control Strategy of the World Health Organization. It remains the most effective way to prevent malaria transmission. A solid understanding of the interrelationship between the vector, the environment and humans leads to the selection and deployment of the most cost-effective and sustainable intervention(s), either individually or combined—the objective being to achieve the maximum possible reduction or local elimination of the disease.	Field demonstration of main vector control tools (LLINs, residual spraying, larvicides)
Malaria stratification and vector control	Endemicity of malaria may vary based on geographic zone or climate. The varying levels of malaria transmission (i.e. year-round transmission vs. seasonal transmission vs. epidemic-prone areas), informs intervention strategies that are most appropriate. Stratification also informs the entomological monitoring techniques and the timing of these.	Selection refining of interventions based on transmission and ecological settings
Identifying between anopheline and culicine	Malaria is transmitted by the adult female anopheles mosquito of different species and complexes. A proper identification of the local malaria vector(s) is a necessary step to tailor interventions appropriately in order to maximize the destruction of local disease transmission. This topic will teach participants to: <ul style="list-style-type: none"> • Know how to identify adult <i>Anopheles</i> mosquitoes from other insects; • Differentiate male and female mosquitoes; • Distinguish the female <i>Anopheles</i> from other female culicines; • Distinguish between the egg, larva and pupa of <i>Anopheles</i> mosquito from other mosquitoes. 	<ul style="list-style-type: none"> • Distinguish various stages of anophelines from culicines • Distinguish males from females
Vector incrimination and	There may be more than one anopheline mosquito in a local area. It is important to know which of these local species	<ul style="list-style-type: none"> • Determination of blood digestion and

Topic	Purpose	Associated Activities	
malaria control (determining that an anopheles mosquito is a vector)	<p>actually transmits malaria and which is a primary vector and secondary vector. Knowledge on these are important to maximize intervention strategy. Participants will have basic understanding on:</p> <ul style="list-style-type: none"> • The methods used to determine that a mosquito species is a malaria vector. • Basic entomological indicators of transmission and how to calculate transmission indices. • Some of the factors that affect malaria transmission. <p>Theoretical submission will also cover methods for determining a malaria vector, including determination of:</p> <ul style="list-style-type: none"> • Contact between the mosquito and humans does occur and that the mosquito feeds on human blood. • The salivary glands of the mosquito contain sporozoites (the stage of the malaria parasite that infects humans). • Relationship, both in time and space, between the mosquito and the local cases of malaria 	<p>ovarian development stage;</p> <ul style="list-style-type: none"> • Demonstration of mosquito dissection and identification of salivary glands and ovaries • Calculation of transmission indices using results from field sampling 	
Sampling of larvae and adult mosquitoes	Larvae	<p>Various mosquito vectors display different larval habitat preferences. Breeding sites can be very diverse, including ponds, lakes, swamps, marshes, rice fields, small rain pools, hoof-prints, car tires, tree holes and plant axils and edge of streams. It is important to know the breeding preferences of the local vectors of malaria in order to implement effective control measures. The reasons for doing larval sampling include:</p> <ul style="list-style-type: none"> ▪ Determination of vector species present in the study area. ▪ Identification of preferred breeding sites for each species. ▪ Determination of the geographical distribution of vectors. ▪ Evaluation of anti-larval measures on larval density. ▪ Collect samples for rearing adults in the insectary. 	<ul style="list-style-type: none"> • Collection of larvae and pupae using dippers, spoons, nets, and pipettes. • Differentiation of immature anophelines from culicines • Calculation of densities
	Adults	<p>Mosquito populations in any locality are made up of different species (individual or as specie/complexes). Species may exhibit varying behaviors which may impact the efficiency of the species as a vector. Variations in behavior may also occur within the same specie due to age or physiological states. Differences may include feeding behavior (indoor or outdoor) and choice of post-feeding resting surface for egg maturation (indoor or outdoor). Various sampling methods have been devised to take into account these differences within the vector populations.</p>	<ul style="list-style-type: none"> • human landing catches, • pyrethrum spray sheet collection, • outdoor resting collection, • hand collection (aspiration) of indoor resting mosquitoes, • exit trap collection •
Preparation, labeling and conservation of mosquito samples	<p>Mosquito samples obtained from larval and adult surveys can be analyzed by a variety of standardized laboratory techniques to obtain important information of the biology of the mosquito species and their role as a malaria vectors. Mosquito samples are generally used for:</p> <ol style="list-style-type: none"> i. Morphological identification of species and species 	<ul style="list-style-type: none"> • Participants will be trained on how samples are prepared, labeled and stored, including time of catch, location, and sample name; 	

Topic	Purpose	Associated Activities
	<p>complexes to assess mosquito vector populations.</p> <ul style="list-style-type: none"> ii. Determination of the gonotrophic state (<i>i.e.</i> abdominal condition of females) to study resting behavior. ii. Determination of physiological age and insemination of females to study the mosquito population longevity and survival. v. Detection of malaria parasites in the mosquitoes to determine sporozoite rates. v. Determination of the origin of the blood meal to study host preferences. vi. Cytogenetic and molecular analyses for sibling species identification and to study genes of interest (<i>e.g.</i> insecticide resistance associated genes). <p>Lab techniques (iv)-(vi) are considered advanced and participants will only be receiving introductory theoretical techniques.</p>	<p>requirements for proper storage and transportation of samples</p> <ul style="list-style-type: none"> • Participants to conduct techniques (i)-(iii).
Basic essentials of rearing mosquitoes in the laboratory	<p>An insectary is important to maintain an adequate supply of laboratory-reared mosquitoes (either fully susceptible and local wild-caught species) for observation, identification and various assessments, such as susceptibility assays to insecticides, estimation of mosquito longevity and feeding habits. This unit will provide knowledge on:</p> <ul style="list-style-type: none"> • The basic characteristics of an insectary. • Basic requirements for rearing larvae and adult anopheline mosquitoes in a laboratory environment. 	<ul style="list-style-type: none"> • Collecting wild-caught species at the larval stage and raising them through adult emergence. Will also include inducing oviposition in the laboratory. •
WHO susceptibility testing and interpretation of results	<p>Resistance of mosquito vectors to insecticides that are used in their control is a growing problem globally. Resistance development threatens the sustainability of malaria control programs. Understanding and knowing the level of susceptibility of local vectors enables the correct selection of pesticide-based intervention and managing local levels of resistance. Depending on country preference and history of use, either or both existing methodologies will be taught (<i>i.e.</i> WHO tube test to estimate susceptibility and CDC bottle assay)</p>	<ul style="list-style-type: none"> • Perform WHO susceptibility tests • Calculate mortality • Abbott's Formula • Interpret results of assay
Cone bioassay tests of insecticide residual efficacy	<p>This technique can be used to evaluate the residual efficacy of insecticide used for residual spraying operations. It can also be used to determine residual efficacy of an insecticide on long-lasting insecticidal nets</p>	<ul style="list-style-type: none"> • Learn how to conduct cone assay on wall surfaces and LLINs • Learn how to use results to calculate knock-down rate/mortality

Outcome: By the end of the trainees will be able to initiate credible and standardized entomological monitoring and reporting to inform vector control decisions.