Entomological part

Objectives

Entomological evaluation will aim at investigating the impact of interventions (combined and alone) on mosquito density per species, aggressivity (Human biting rates) and infectivity rate of the vectors (by using standard CSP/PCR methods). These indicators will serve at evaluating the intensity of malaria transmission by comparing the Entomological Inoculation rates (i.e. number of infected bites per man per year) between treated arms. Additional objectives will aim at investigating the impact of each intervention on vector behaviour, mosquito longevity (age grading), and resistance selection**.**

Assessment of the quality of the implementation

- When possible the quality of the treatments should be carefully assessed. For mosquito nets, this can be achieving by quantifying the insecticide content on nets using standard chemical analysis (e.g. CIPAC methods). For IRS, papers (Whatman® No.1) should be attached to the walls of randomly selected houses and removed after the spray campaign and assayed for pesticide residue.
- Residual activity of insecticide (e.g. mosquito nets and IRS) should be checked regularly by conducting in situ WHO cone bioassays using well characterized mosquito strain (at least susceptible reference strain).

Entomological measurement

- A minimum of 4 entomological surveys should be done in each village/cluster per year.
- Different methods of measuring population abundance may be used, each with advantages and limitations. Monitoring should be carried out in untreated "sentinel rooms", which are maintained for this purpose throughout the study.
- Human landing Catch is known to be the best technique for collecting anthrophilic mosquitoes. Catchers may be required to work within and outside houses to assess indoor and outdoor biting rates (exo-endophagy). A sufficient number of collection sites should be used and kept throughout the study. For village having around 50-60 houses, select a minimum of 4 houses at random within the village and conduct of mosquito collection inside and outside the house during 2 consecutive nights (16 human night captures). A minimum distance of 100 meter should be adopted between two collection sites to be representative of the mosquito density in the village. Prophylaxis and/or drug delivery to mosquito collectors should be done according to the recommendation of the national ethical committee. Regular supervision of mosquito collectors (QC) should be done to ensure that mosquito sampling and collection strictly follow the procedures.
- Due to ethical constraints, other collection methods can be used such as light traps, window trap, PSC and pit traps. It is recommended that at least 2 methods be employed to allow for calibration between different studies in different countries.
- For PSC, a Minimum number of 10 houses should be selected at random during each survey.
- For Window traps and pit traps, a minimum of 4 sentinel sites should be required per treated arms.

- When using light traps, device should be used in occupied room without treated nets.

Resistance assessment

- The objective is to characterize the baseline resistance of the mosquito population and to follow up the evolution of resistance after the implementation of the interventions, alone and combined.
- Bioassays should be done at least twice per year using same insecticides used during the trial, at the WHO diagnostic dosage, according to WHO guidelines. Synergists (enzyme inhibitors) can be used to understand better the metabolic basis of resistance. Bioassays should be done preferably on adult mosquitoes coming from larval collection (F0). If not, bioassays should be done using F1 progeny of field collected adult mosquitoes). A minimum of 30 mosquitoes from the control should be genotyped for species, molecular forms (if required) and know resistant genes (kdr / ace.1) using standard PCR assays. Equivalent number of dead and alive mosquitoes exposed to insecticides can be genotyped to assess the phenotypic resistance.

Age Grading

- Physiological status and parity rates should be measured at each survey taking into account natural fluctuation in age structure. New methods of age grading are urgently needed to replace existing techniques.

Infectivity Rates & blood meal status

- Diagnosis of *Plasmodium* species is generally done by ELISA test to detect circum-sporozoite protein (CSP) of *Plasmodium falciparum* or *P. vivax* (using species specific monoclonal antibody).
- Blood-meal identification of individual mosquitoes collected in PSC, window traps and pit traps should be established using standard ELISA technique.

General mosquito processing

- Species ID by morphological techniques
- Physiological status (fed/unfed,etc.)
- Parity rate for unfed mosquitoes
- Preservation of mosquitoes
    - Desiccation for molecular processes
        - Species ID
        - Molecular form
        - Sporozoite:blood meal ELISA
        - Resistance genes (new methods are now available for detection of target site and are being developed for metabolic resistance)
    - Fresh/frozen
        - Biochemical assays