Vector-borne Diseases Control Programme
Department Of Public Health
Ministry of Health
Royal Government of Bhutan
2021

National Insecticide Resistance Monitoring and Management Plan 2021

Abbreviation

ACD	Active Case Detection
API	Annual Parasitic Index
APLMA	Asia Pacific Leaders Malaria Alliance
APMEN	Asia Pacific Malaria Elimination Network
BMHC	Bhutan Medical and Health Council
CDC	Centres for Disease Control
DHO	District Health Officer
DMS	Department of Medical Services/District Malaria Supervisor
GFATM	Global Fund for AIDS, Tuberculosis and Malaria
HISP	Health Information Systems Programmes
IEC	Information, Education and Communication
IRS	Insecticide Residual Spray/Indoor Residual Spray
IRM	Insecticide Resistance Monitoring
IT	Information Technology
IVM	Integrated Vector Management
LLIN	Long Lasting Insecticidal Net
NVBDCP	National Vector Borne Disease Control Programme
PCR	Polymerase Chain Reaction
PF	*Plasmodium falciparum*
PV	*Plasmodium vivax*
QC	Quality Control
SOP	Standard Operating Procedure
TAGME	Technical Advisory Group for Malaria Elimination
UN	United Nations
VDCP	Vector Borne Disease Control Programme
WHA	World Health Assembly
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

Acknowledgements

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The development of the plan was led by Dr Pradeep Srivastava, Consultant, supported by APLMA-APMEN. Mr. Rinzin Namgay, Chief Entomologist, VDCP, Bhutan, and Dr Kinley Penjor and Dr Tobgyel Drukpa from the National Malaria Programme have been supportive in providing feedback, especially on the feasibility of proposed solutions. Their support during the preparation of the document is deeply valued.

The APMEN Vector Control Working Group is acknowledged for its continuous support and expert guidance in the development of this national plan.

Supported by:

aplma ASIA PACIFIC LEADERS MALARIA ALLIANCE

apmen ASIA PACIFIC MALARIA ELIMINATION NETWORK

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Executive Summary

Malaria and other vector-borne diseases (VBDs) are a major concern in public health programmes. Global Vector Control Response (GVCR) 2017–2030, adopted by World Health Assembly in 2017, provides strategic guidance for strengthening key elements for preventing mortality, and control and elimination of vector-borne diseases.

Insecticide has been an important tool in malaria control programmes, but effective malaria vector control is threatened by increasing insecticide resistance. Delay in corrective measures or failure to mitigate insecticide resistance is likely to hamper malaria elimination. The Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM) was released in 2012, which is a guiding document for countries to address the issue of insecticide resistance. The framework for formulation of the National plan for Insecticide Resistance Monitoring is another advisory document of WHO to facilitate the countries in making a national plan for IRM.

The document has components on situational analysis, implementation framework and financial implications including a calendar of physical activities.

The situational analysis includes the demographic profile of the country, epidemiological situation of malaria in Bhutan, and stratification at the sub-district level, known as Gewog. Bhutan has already been stratified into three categories namely **no risk** areas, **potential risk** areas and **low-risk** areas. The major malaria vectors and other anopheline species encountered in Bhutan have also been detailed in the document. *An. culicifacies* has been playing the role of major malaria vector in Bhutan but the record of *An. pseudowillmori* indicates its suspected role in malaria transmission. The presence of *An. dirus* in forest fringe areas of Sarpang district indicates the influence of a third vector.

The vector control measures started in the sixties with DDT, have now been switched over to synthetic pyrethroids. Long-lasting insecticidal nets have been a major tool in malaria-endemic districts. Though there have been constraints of human resources for conducting entomological surveillance, evaluation of vector control tools has somehow been undertaken. The situation analysis also includes the partners' contribution, especially on entomological surveillance and insecticide resistance monitoring. The issue of entomological surveillance and insecticide resistance monitoring along with the gaps in human resources have been flagged in this document. The challenges highlighted are not very specific to Bhutan but are prevailing across many countries, as they are also facing similar challenges.

The document also covers an implementation framework that describes that the prime goal and objective of insecticide resistance monitoring is to update the insecticide resistance status and suggest any alternatives to delay the resistance if it is recorded. The process of insecticide resistance

monitoring has also been detailed and the plan has been explained to select the sentinel sites within the feasible resources. In consultation with the programme, it has been worked out that out of eight endemic districts, five districts will be taken for insecticide resistance monitoring and within the districts, one sub-districts with one representative village will be taken up as a sentinel site for the collection of mosquitoes, except one district which will have 4 sentinel sites.

Human resource constraints have also been flagged and taken into consideration. The plan has been based on the available six-person team including one central entomologist, one technician, three insect collectors, and one driver who will visit the identified five districts during the period from June to October. Accordingly, the calendar of activities has been made so that the six people can visit the field, stay overnight for 4 to 5 days, and perform the susceptibility test to cover the district identified for insecticide resistance monitoring.

The budget for honorarium, transportation and contingency has been worked out, excluding the salary component. It has also been proposed in the document that the whole plan will continue for three years to monitor the insecticide resistance in the sentinel sites to map out the status.

The plan to compile reports and submission to committee has also been described. The documentary evidence generated will be placed for perusal and decision-making. The decisions taken by the committee will be disseminated in subsequent months to the implementing units.

The document is expected to guide the field staff while performing the tests. The methodology of the WHO protocol has also been detailed in this document.

1. Situation analysis

Epidemiology of malaria

Bhutan is a mountainous country spread over 38,394 square kilometers. The southern area of the country is flat land with 70 percent forest cover. Bhutan has borders with India in the west, south and east, and with China (Tibet) in the north. The land area in the southern foothills experiences hot and humid conditions, whereas central valley areas have a cold climate, and the northern mountainous area has an extreme cold with virtually snow-capped mountains. The rainfall varies in different regions with higher annual precipitation of 2000-5000mm in the south, 600-800 mm in the central region and about 75-200mm in the north. The vector-borne disease like malaria and dengue are prevalent in southern foothill areas due to favorable climatic factors. However, global warming may increase the vulnerability of some more areas.

Bhutan comprises 20 districts which are further divided into 205 *gewogs* (sub-districts) (**Figure 1**). Each *Gewog* covers 5-8 c*hiwogs* (sub-block) consisting of few villages by each chiwog.

The malaria eradication programme in Bhutan started in 1964 and made significant progress except during the malaria epidemic in the 1990s. Further, with funding support from Global Fund for AIDS, Tuberculosis & Malaria (GFATM), malaria morbidity and mortality in the country were reduced. The integrated Vector-borne Diseases Control Programme is responsible for planning and coordinating the prevention and control of vector-borne diseases in the country.

The southern districts of Bhutan are malaria-endemic and both *Plasmodium falciparum* and *Plasmodium vivax* malaria parasite species are found in Bhutan. The maximum cases of 39,852 were reported in 1994. However, it was reduced to 436 cases by 2010 and just 2 indigenous cases of the total of 42 cases reported in 2019. During the year 2020, an upsurge was recorded with 22 indigenous cases out of total of 54 cases. The seasonal trend of malaria indicates two-peak in a year, first before monsoon and the second in the end. Reporting of malaria cases from **seven southern** districts of Bhutan, bordering India, indicates cross-border malaria and requires monitoring of each case to classify indigenous or imported. The receptivity to malaria transmission increases in the monsoon period, from April to September.

The country was stratified earlier at the districts level into 4 categories *viz.,* high, low, potential and no transmission zones. The criteria for such stratification were based on reported malaria cases, the inter-country border with India, vector prevalence and climatic factors. However, based on the low number of malaria cases, the stratification has been done to Gewog (sub-district) level in different categories:

- ***Category-1 No Risk*** – Gewogs free of malaria transmission with no reports of local malaria transmission due to high altitude and absence of vectors.

- ***Category-2 Potential risk*** – Gewogs having, both, receptivity of ecosystem and vulnerability for transmission of malaria (vector present at least during summer, presence of mega projects with high volume of migrants from malaria-endemic areas) but without reports of local malaria transmission within the last three years.

- ***Category-3 Low risk*** – Gewogs that have both high receptivity and vulnerability and evidence of local malaria transmission within the last three years or share a direct international border with India. All gewogs with common and porous border with malaria-endemic states of India that lie within mosquito flight range and have a high number of population influx and reflux from across the border.

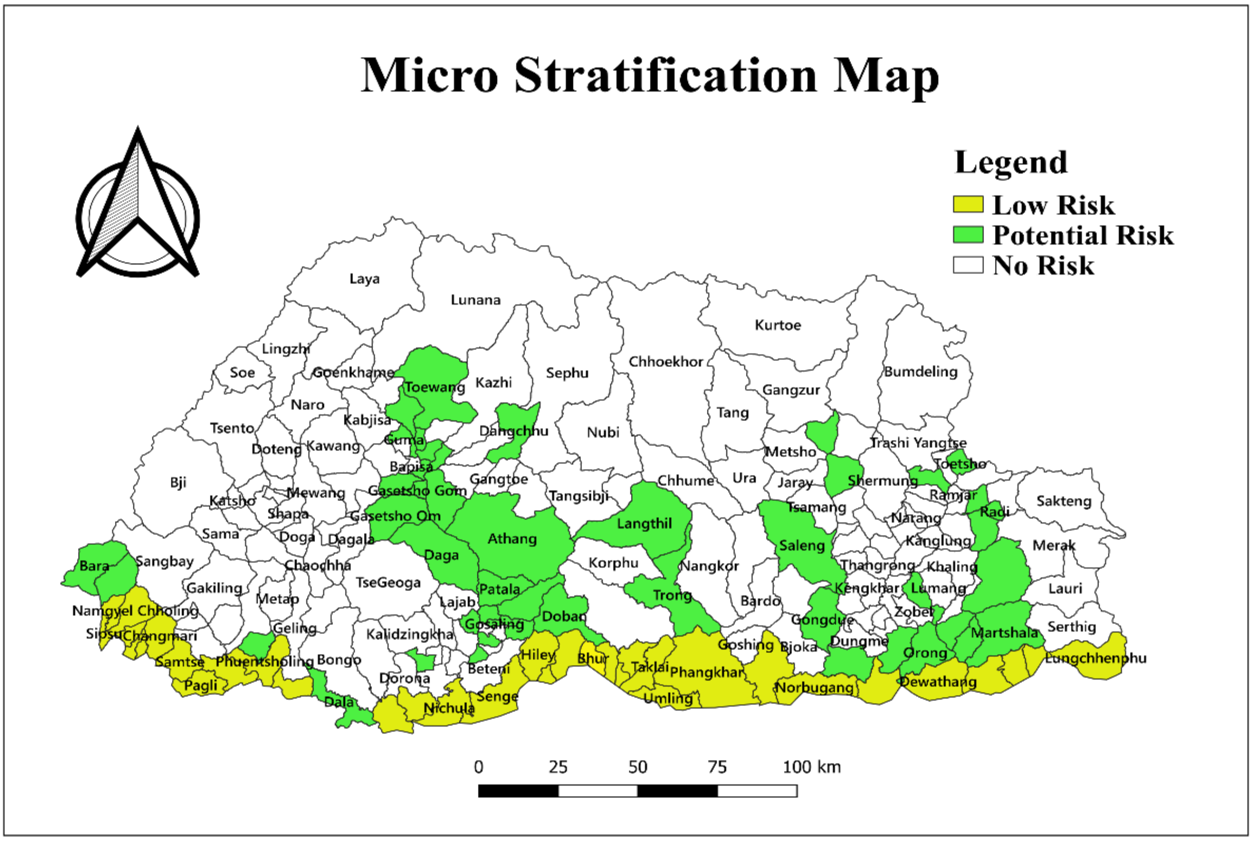


Figure 1: malaria risk areas in Bhutan

Anophelines and Malaria Vectors in Bhutan:

Regular entomological surveillance is a challenge due to entomological infrastructure as well as difficult terrain, however, as per the record, a total of 32 anopheline species have been recorded. *An. pseudowillmori* and *An. culicifacies* are suspected to be the potential malaria vector species for malaria transmission in Bhutan. In October 2019, *An. dirus* was found in forest fringe areas in Sarpang Bhutan. The anophelines encountered in low lying areas *are An. pseudowillmori, An. vagus, An. subpictus, An. culicifacies, An. jamesii, An. pseudojamesii, An. nevipes, An. annularis, An. philippinensis, An. kochi, An. peditaeniatus, An. aconitus, An. barbirostris, An. barbumbrosus and An. umbrosus.* The mountain species encountered are *An. maculatus, An. willmori, An. lindesayii, An. baileyii, An. aberrans and An. bengalensis, An. interruptus* and *An. barianensis*.

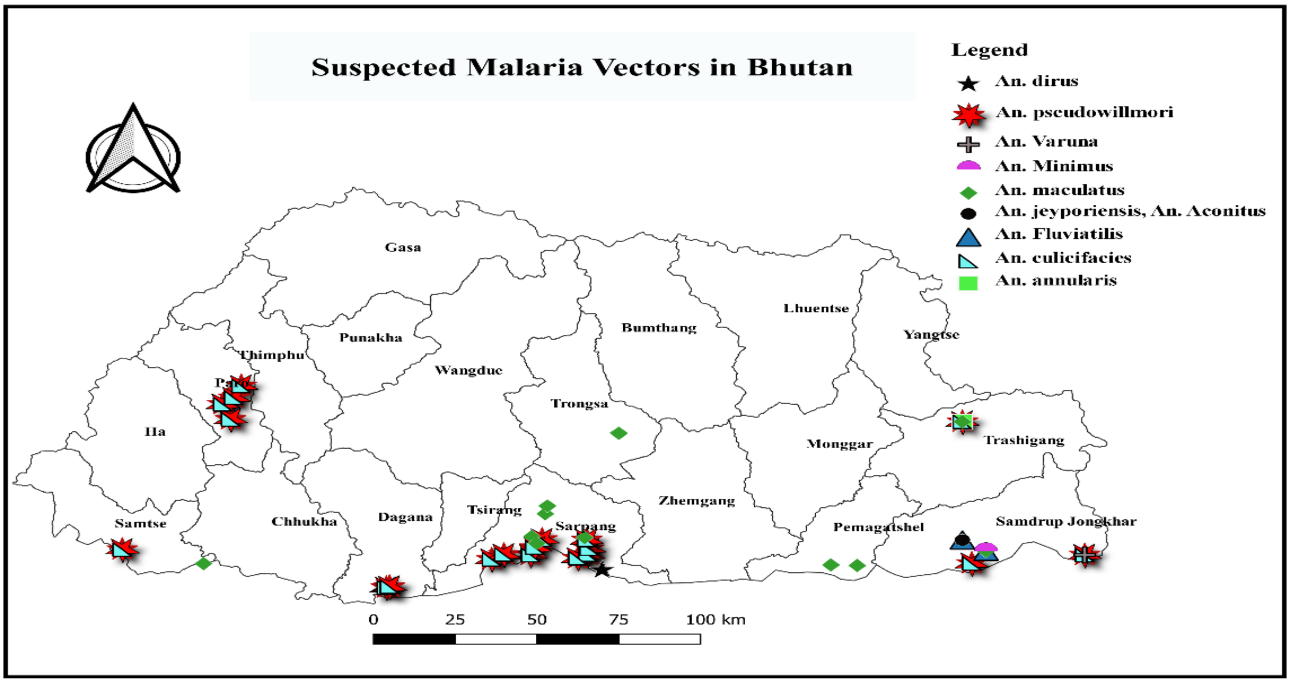


Figure 2: Distribution of malaria suspected vectors in Bhutan

- Earlier recorded species viz., *An. minimus, An. fluviatilis, An. dirus, An. tessellatus, and An. jaiporeiensis* are not encountered during the past few years.
- *An. minimus, An. fluviatilis* and *An. Dirus,* earlier presumed to play a role in malaria transmission in Bhutan, are not encountered in recent past, except *An. dirus* immature stages which were found in forested areas of Sarpang in 2019.
- The abundance of *An. culicifacies* and possibly *An. pseudowillmori* are suspected to be vectors because of their endo and exophagic and anthropophilic behavior.
- Foci investigations also revealed the association of cases mostly with prevalence of *An. culicifacies and/*or *An. pseudowillmori*

Vector Control Interventions

- The main malaria control strategies consist of LLINs and two rounds of focal IRS, carried out based on the micro stratification at the Chiwog level. This geographical unit is considered as foci for active, residual non-active and cleared transmission to plan and implement prevention and control activities.
- Under the malaria control programme, DDT was the main vector control measure from 1960s to 1990s, which, however, was replaced with synthetic pyrethroid in 1995 due to reported resistance in *An. culicifacies* and other malaria vector species in India.
- Long-lasting insecticidal nets (LLINs) were introduced in 2006 and continues to date with the strategy of its replacement every three years. IRS is now confined to stratified areas with active local transmission or areas with high vulnerability.
- The districts and sub-districts reporting zero indigenous malaria cases during the past three or more years can withdraw IRS, but in case of reported indigenous case focal IRS is allowed.
- Other measures are source reduction and anti-larval measures. Larval source management was done at the community level in rural areas through Community Action Groups (CAG) in all malaria risk Chiwogs. Anti-larval measures, with chemical (temephos), in urban areas are withdrawn since 2010, considering its cost-effectiveness. Moreover, in urban areas no *Anopheline* breeding and malaria cases have been recorded till 2019. In 2020 pyriproxyfen and temephos granules were used in selective anopheline breeding sources.
- Environmental management is promoted through CAGs with emphasis on the prevention of mosquito breeding sites near human settlements.

Monitoring of Vector Control Interventions

The other interventions like community mobilization activities and monitoring are conducted in all foci but more frequently in active foci. The programme activities are monitored to check:

- Distribution of LLINs, anti-malaria drugs and rapid diagnostic tests (RDTs) kits in all foci including non-receptive foci.
- Implementation of Indoor Residual Spray (IRS) in active, residual foci and in receptive foci cleared but situated along malaria-endemic Indian borders.
- Entomological monitoring is done as part of the focus investigation after the detection of indigenous cases. Such entomological monitoring includes:
    - Rapid assessment of species prevalence and density
    - Parity rate
    - Infection in vector species
    - Mapping of breeding sources within one kilometer radius of the index case

Evaluation of vector control tools

- Bio-efficacy of insecticide used in LLINs and IRS through bioassay. The tests are done in 3 sentinel sites for LLIN in quarters 1 & 3 and for IRS in quarters 2 & 4. It was observed that, from 2019 onwards, the efficacy on sprayed walls was reduced as the observed mortality rates were even below 50% in tests.
- The development of a resistance management plan, under the programme, to facilitate decision on the use of appropriate insecticide was considered in 2021.

Registration of Insecticides

The “Pesticides Act of Bhutan, 2000" is in force to regulate the safe use and handling of chemicals to prevent public health and environmental hazards. The Act describes "Pesticide" means any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feeds, or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of it, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

The objectives of this act are:

- To ensure that integrated *pest* management is pursued, limiting the use of pesticides as the last resort.
- To ensure that only appropriate types and quality of pesticides are introduced into Bhutan
- To ensure that pesticides are effective when used as recommended
- To minimize deleterious effects to human beings and the environment consequent to the application of pesticides; and
- To enable privatization of sale of pesticides as and when required.

The import, manufacture, sale, and use are governed as per the authorization accorded under the act and only the authorized person/manufacturers/ suppliers, that are registered, may sell or supply.

Entomological surveillance, including insecticide resistance monitoring

- Entomological surveillance for malaria and other VBDs is conducted by the team working for malaria. Collection of adult mosquitoes is done through cattle biting collections, morning resting collection and light trap. However, its performance is hampered due to multitasking at the district and health facility levels.
- Larval surveys are conducted to map with GPS for specific species breeding sites.
- *An. interruptus* and *An. barianensis* –the species of higher altitude were recorded first time in Bhutan. *Anopheles (Cel.) dirus* breeding was confirmed for the first time in Bhutan in 2019, under thick canopy forest covers.
- A total of 128 mosquito species have been recorded in Bhutan which includes 32 Anopheles species, 51 *Aedes* species, 33 *Culex* species and 12 other species belonging to various genera such as *Malaya, Topomyia, Orthpodomyia, Triptriroides* and *Toxorhynchites*. In 2021, there is a plan to introduce online entomological surveillance recording and data management with the help of WHO, SEARO and HISP-India.
- Besides entomological surveillance, insecticide susceptibility tests are undertaken in the sentinel sites using WHO discriminating dosages.
- The data of 2019-2020 from sentinel sites have revealed moderate pyrethroids resistance in *An. pseudowillmori* and *An. culicifacies.* These need to be confirmed by using intensity dosages (5x & 10x) and synergist-insecticide tests by 2021.
- To understand the insecticide resistance pattern in *An. pseudowillmori, the species* have been sent to Chiang Mai University, Thailand for PCR detection of Kdr genes.
- Monitoring insecticide resistance development in the vector species as routine surveillance had remained one major challenge for the national programme owing to resource and logistical problems.

Evidence and Knowledge Gaps through Operational Research

The operational research related to malaria elimination has been undertaken and based on the evidence the strategies are adopted. The operational research includes different fields, however, insecticidal efficacy, fauna survey and resistance studies are listed below:

- Quarterly Bioassay Test and Insecticide Susceptibility Test
- Mosquito and other vectors in Bhutan www.elsevier.com/locate/actatropica
- Insecticide efficacy studies on wall and LLIN in 2017-2018
- Mosquito species prevalence surveillance

Human Resources

- Human resource development through training on vector control and surveillance at the national level has been conducted by the Entomology Unit of the programme.
- The training for IRS and field surveillance by spray squads and insect collectors are trained on spray techniques and mosquito sampling methods at the health facility level are trained by district and field malaria staff themselves.
- In addition to WHO and APMEN Fellowships and short-term training on entomology and vector control abroad, the programme conducts in-country training through the Global fund support
- At the field level, regular supervision on vector control and surveillance is done from the district but the programme also makes regular supervisory visits in high-risk areas.
- To do the above activities, skilled HR is required, and they should be retained.

Partner Contributions

National and international collaborative partnerships continued, especially during the elimination phase, due to which the malaria programme could achieve success in reducing and sustaining fewer malaria cases. The main international collaborating partners are WHO, the Government of India and GFATM, they are the main funding and technical partners of the VDCP programme. The continued support from GFATM support had significant contributions in realizing the objectives of malaria control and elimination in Bhutan. The support of APMEN to Bhutan in conducting operational research and capacity building has led the programme to improve significantly. Some of the outcomes of APMEN support through fellowship in vector surveillance and malaria mapping includes GIS mapping and micro-stratification of malaria transmission areas and vector surveillance in the country, besides technical assistance. The development of the Insecticide Resistance Management Plan is also supported by APMEN.

Current Constraints

- **Entomological surveillance:** The bio-efficacy tests of LLIN and IRS are done routinely in sentinel sites. During the survey, bionomics of *Anopheles* species, its prevalence and densities are also monitored. Adult resting mosquitoes are collected for the determination of indoor density and assess its density in human dwellings. Larval surveys for density and mapping of the breeding site are also done during the survey. However, these activities are confined to sentinel sites in Sarpang district only. The rest of the districts have limited numbers of Malaria Technicians in health facilities and due to multitasking, the implementation of entomological surveillance is affected even though the activities are mandated. The Programme's Entomology Unit undertakes ad-hoc vector surveillance in other districts which is limited due to human resources and other programme priorities.

- **Insecticide Resistance:** One of the main issues on vector control is insecticide resistance monitoring. The lack of historical records on resistance status of vector species against insecticides used under vector-borne diseases control programme is a challenge in mapping the resistance status. Further non-availability of scientific data on monitoring of resistance intensity and use of synergists, such as piperonyl butoxide (PBO), with prevailing resistance, is the greater challenge. These challenges increase manifold when coupled with non-availability of logistics such as insecticide-impregnated papers, test kits and capacity of human resources to perform the tests. To address such challenges and bridge the gaps, arrangements of necessary logistics with the support of Global Fund were made in 2021. The activities are undergoing in 4 sentinel sites of Sarpang district only.

- **Human resource:** The gap in human resources is often not given due attention which results in a serious threat towards non-production of scientific and consistent entomological data. The existing human resource includes only two entomologists in the programme assisted by two Malaria Technicians and 3 Insect Collectors. Most of the health facilities have just one Malaria Technicians who are overburdened with daily laboratory malaria screening, dengue vector surveillance, routine malaria control with IRS. In addition, they do case and focus investigations, follow-up of cases and reporting as per the format required in malaria elimination programme. The problem due to shortage of human resource is slowly getting aggravated as the senior malaria workers superannuate year after year and no new recruitment is being done. Non-filling of such posts has resulted that some of the health facilities located in high malaria risk districts do not have Malaria Technicians now. However, some corrective measures have been initiated to train a new batch of multitasked Entomology Technicians (3 years course) under the University of Medical Sciences of Bhutan but it will take a few years to produce 1st batch.

2. Implementation Framework

Goals and objectives

To conduct susceptibility test among malaria vectors and establish insecticide resistance status and suggest alternatives to delay resistance if any.

Insecticide resistance monitoring

- **Sentinel sites** will be selected after preliminary survey of mosquito density in different seasons
- **Mosquitoes to be tested**

  Female vector mosquitoes need to be subjected to susceptibility tests. It is recommended to use 3-5 days old adult female mosquitoes that are non-blood fed (*i.e.,* sugar-fed and starved for about 6 hours) to nullify any influence of age and physiological status on mortality. Ideally, the larval collections are suggested and so that the adults who emerged as first generation (F1) will be of the same age, and these are subjected to tests.

  It is important to record the type of breeding site (*e.g.,* rice field, rainwater collection, irrigation channel, well or others) and the global positioning system (GPS) coordinates from which the larval collection was made.

  If the larval collection is difficult or not possible, the adult-fed females will be collected and reared in the laboratory up to F1 generation. This is a difficult and time taking process and requires good insectary with the laboratory. Therefore, in the programme usually field-caught mosquitoes are tested.

  In the case of field-caught mosquitoes following points will have to be observed:

  - Selection of only unfed females for the test.
  - Females will be fed with sugar water and then starved for 4-5 hours before the tests.
  - Morphological identification of species will be done after insecticide susceptibility tests.
  - Dead mosquitoes will be preserved for reference.
  - Support of international laboratories will be taken to distinguish individual members of mosquito species complexes using simple PCR-based assays.

Sample size:

The standard procedure as per WHO will be followed using WHO adult susceptibility test kit, insecticide-impregnated papers and control papers impregnated with the appropriate carrier oil only (without insecticide). The following points will be considered during execution:

- A total number of 120–150 adult female mosquitoes of a given species are required to conduct a single set of WHO insecticide susceptibility tests (100 for exposure to the insecticide and 50 for control)

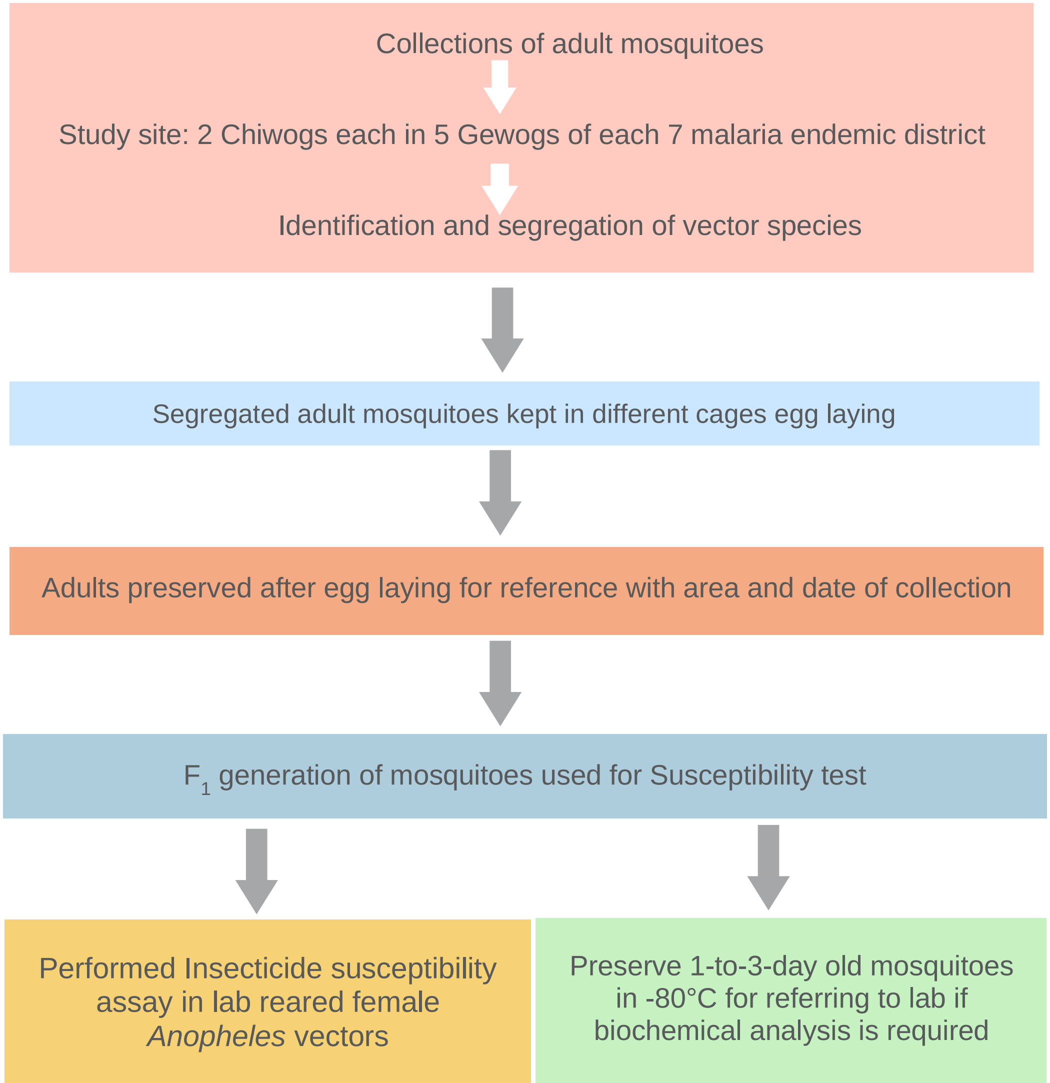
In the case of wild-caught mosquitoes especially outdoor biters (exophagic) and outdoor resters (exophilic), the numbers are often inadequate for performing the tests. In such a situation, the number of replicates can be reduced but will have to be repeated twice or thrice in the same area

The males are usually smaller, have a shorter life expectancy and are more fragile than females, therefore tend to have higher control mortalities.

- The test will be replicated as:
  - For insecticide exposure 4-5 replicates with around 20–25 mosquitoes
  - For the control test, 2 replicates with around 20–25 mosquitoes.
  - For different insecticides separate tests will be conducted

Insecticide Susceptibility Assay:

The plan proposed for conducting susceptibility tests is tentative and may be amended depending on the availability of human and financial resources. The schematic flow chart to cover all endemic districts is depicted below:



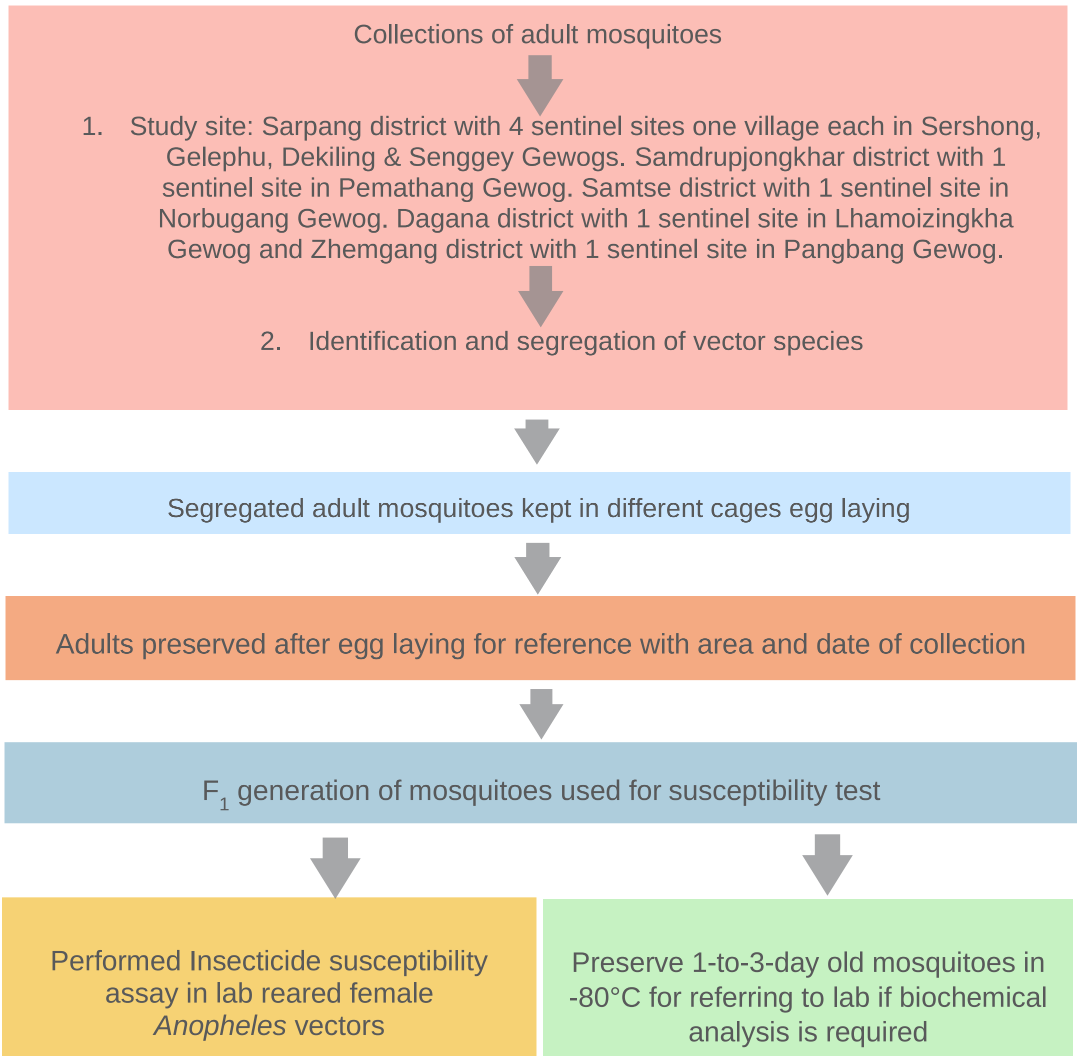
Feasible Amended Plan for Insecticide Resistance Monitoring

The sentinel sites recommended in the above chart are not feasible with existing human and other resources, therefore the amended feasible plan as per suggestions of programme officials is described below. The sentinel sites in priority districts are suggested as follows:

1. **Sarpang district** with 4 sentinel sites (Sershong Gewog, Gelephu Gewog, Dekiling Gewog & Senggey Gewog).
2. **Samdrupjongkhar district** with 1 sentinel site in Pemathang Gewog.
3. **Samtse district** with 1 sentinel site in Norbugang Gewog
4. **Dagana district** with 1 sentinel site in Lhamoizingkha Gewog.
5. **Zhemgang district** with 1 sentinel site in Pangbang

Considering the above feasible solution, the schematic flow chart to cover identified districts is depicted below:

Feasible Schematic Chart for Insecticide Resistance Monitoring in Bhutan



Insecticide Susceptibility Tests – Methodology:

- WHO country office, Bhutan will be requested to supply the adult mosquito susceptibility kits (minimum 4 sets) and insecticide-impregnated along with respective control papers. These kits and papers are supplied by Universiti Sains Malaysia, Malaysia (www.usm.my).
- Adult insecticide susceptibility tests will be performed according to the WHO suceptibilty test kit and method (WHO, 2016). The points to be followed as per the WHO guidelines are reproduced in box below for ready reference.

1. The investigator puts on gloves. Six sheets of clean white paper (12 × 15 cm), rolled into a cylinder shape, are inserted into six holding tubes (with the green dot), one per tube, and fastened into position against the wall of the tube with a steel spring wire clip. The slide unit is attached to the tubes at the other end.
2. Ideally, 120–150 active female mosquitoes are aspirated (in batches) from a mosquito cage into the six green-dotted holding tubes through the filling hole in the slide, to give six replicate samples of 20–25 mosquitoes per tube.
3. Once the mosquitoes have been transferred, the slide unit is closed, and the holding tubes are set in an upright position for 1 hour. At the end of this time, any moribund mosquitoes (i.e., those unable to fly) and dead mosquitoes are removed.
4. The investigator inserts one oil-treated paper (the control) into each of two yellow-dotted tubes, ensuring that the label of the paper is visible on the outside of the tube. The paper is fastened with a copper clip and the tube is closed with a screw cap.
5. Four exposure tubes with red dots are prepared in much the same way as the yellow-dotted tubes. Each of the four red-dotted exposure tubes is lined with a sheet of insecticide-impregnated paper such that the print label is visible on the outside. Each paper is then fastened into its position against the wall with a copper spring-wire clip and the tube is closed with a screw cap.
6. The empty exposure tubes are attached to the vacant position on the slides and, with the slide unit open, the mosquitoes are blown gently into the exposure tubes. Once all the mosquitoes are in the exposure tubes, the slide unit is closed (usually a cotton wool plug is inserted into the hole to lock the slide) and the holding tubes are detached and set aside. The investigator now removes the gloves

7. Mosquitoes are kept in the exposure tubes, which are set in a vertical position with the mesh-screen end uppermost, for a period of 1 hour (unless otherwise specified). The tubes are placed in an area of reduced lighting or covered with cardboard discs to reduce light intensity and to discourage test mosquitoes from resting on the mesh- screen lid.
8. At the end of the 1-hour exposure period, the mosquitoes are transferred back to the holding tubes by reversing the procedure outlined in Step 6. The exposure tubes are detached from the slide units. A pad of a cotton wool soaked in 10% sugar water is placed on the mesh-screen end of the holding tubes.
9. Mosquitoes are maintained in the holding tubes for 24 hours (or longer for slow-acting compounds). During this time, it is important to keep the holding tubes in a shady, sheltered place in the laboratory or in a chamber maintained at 27 °C ± 2 °C temperature and 75% ± 10% relative humidity. Temperature and humidity should be recorded during the recovery period.
10. At the end of recovery period (*i.e.*, 24 hours post-exposure or longer for slow-acting compounds), the number of dead mosquitoes is counted and recorded. An adult mosquito is considered to be alive if it is able to fly, regardless of the number of legs remaining. Any knocked down mosquitoes, whether or not they have lost legs or wings, are considered moribund and are counted as dead. A mosquito is classified as dead or knocked down if it is immobile or unable to stand or take off. [Extracted from WHO Manual]

- Mosquitoes will be exposed to four different classes of insecticides with its control paper such as DDT 4% with Risella oil, Malathion 0.5% with olive oil and Deltamethrin 0.05% and other synthetic pyrethroids with silicone oil. Since Bhutan has also used Bendiocarb for IRS, the carbamate paper-with control will also be required for testing.

Fig 3: WHO Tube Test Kit

Fig 4: Aspirating Mosquitoes from the cage

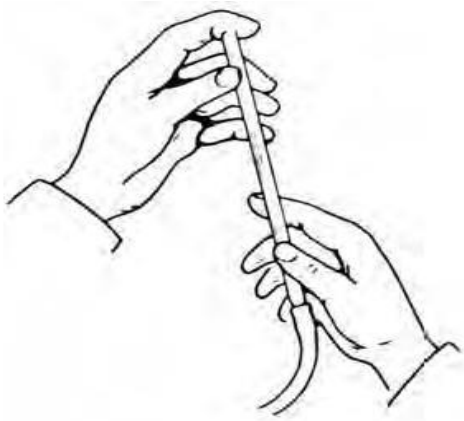


Fig 5: Releasing of mosquitoes to the holding tube

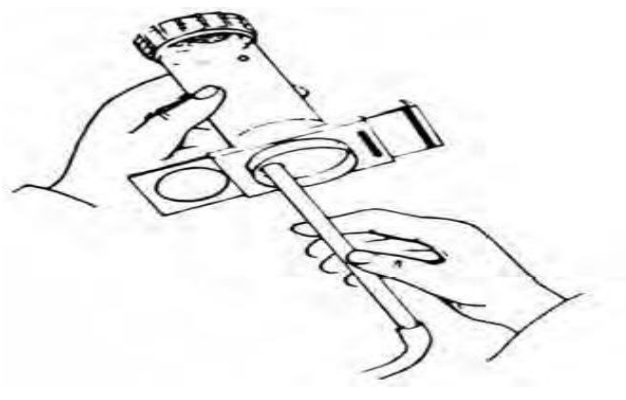


Fig 6: Lining the tubes with impregnated papers

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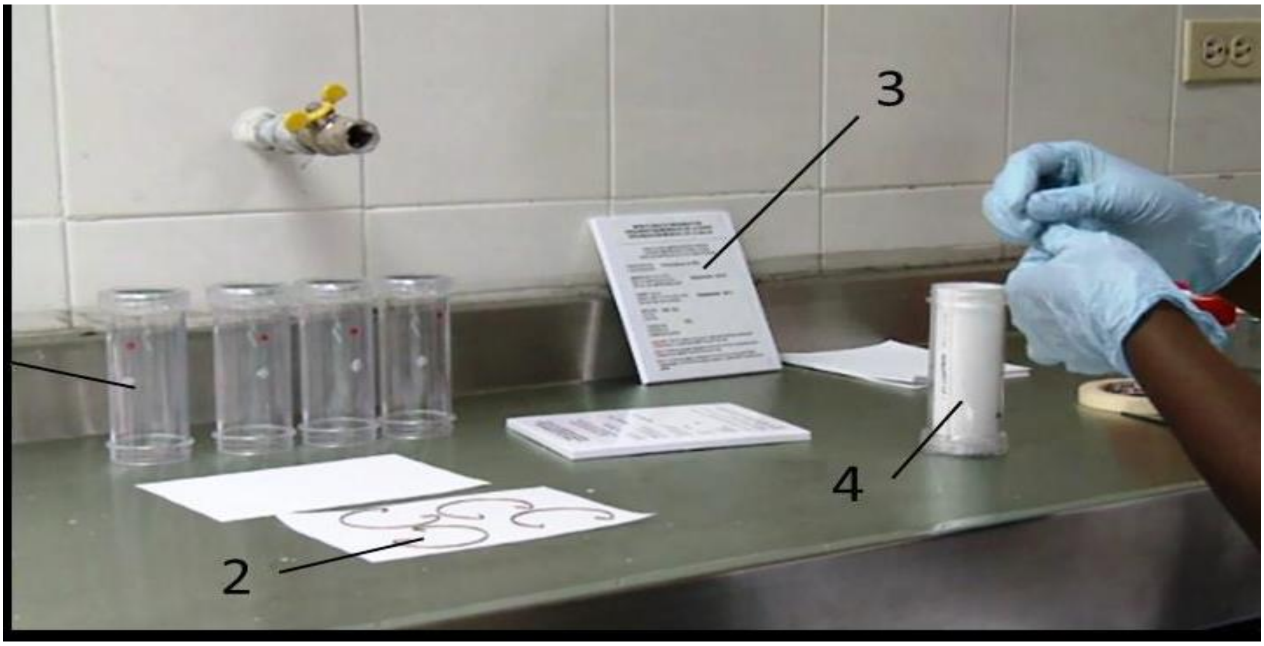

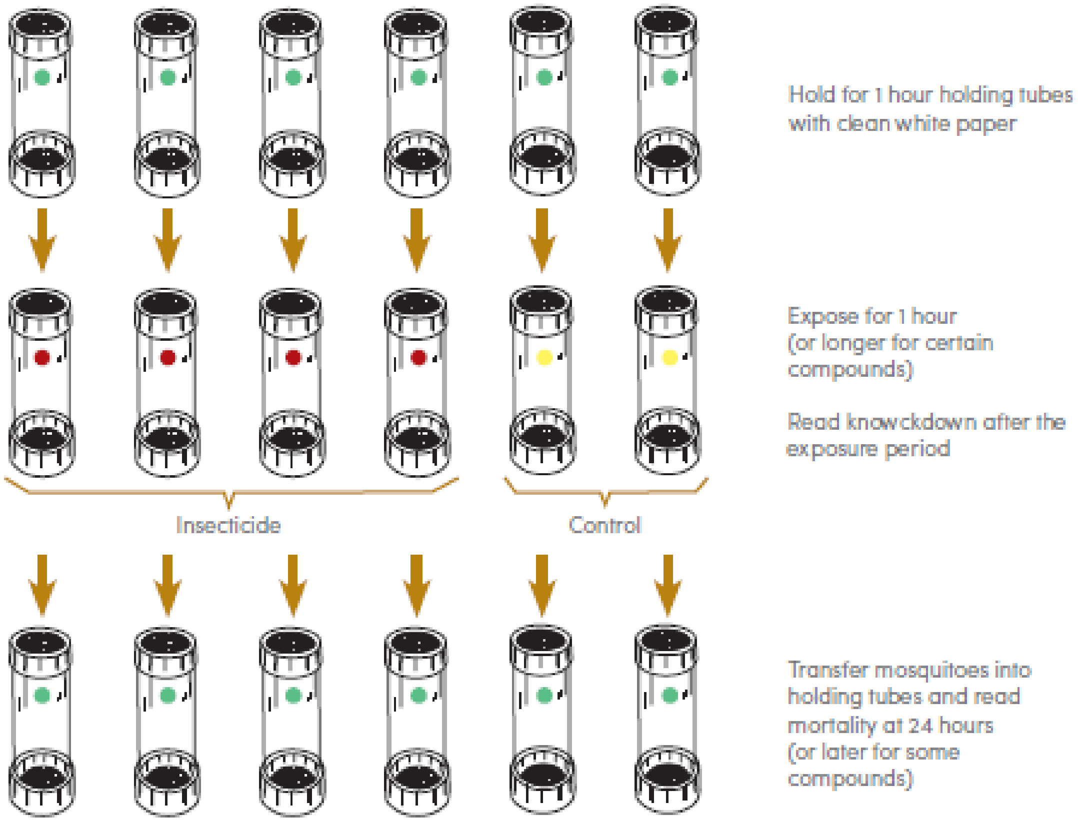

Fig 8: Test Procedure (Extracted from WHO Manual)

- After 24 hours of holding, percent (%) mortality was calculated in both control and test replicates. Percent mortality was calculated by applying the above mentioned formula.

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times 100$$

- If the mortality in control replicates, calculated between 5-20 %, the corrected % mortality is calculated by applying Abbott's formula (Abbott, 1925). In case if control mortality is more than 20% the test must be discarded and repeated.

$$\text{Corrected mortality} = \frac{(\%\ \text{observed mortality} - \%\ \text{control mortality})}{(100 - \%\ \text{control mortality})} \times 100$$

- The susceptibility or resistance status in mosquitoes will be reported based on the WHO criteria. The mortality between 98-100% indicates susceptibility (S), mortality between 90-97% indicated possible resistance (PR) and mortality below 90% indicates resistance (R) (WHO, 2016).

Interpretation of Data:

- After 24 hours of holding, percent (%) mortality was calculated in both control and test replicates. Percent mortality was calculated by applying the above mentioned formula.

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times 100$$

- If the mortality in control replicates, calculated between 5-20 %, the corrected % mortality was calculated by applying Abbott's formula (Abbott, 1925). In case if control mortality is more than 20% the test must be discarded and repeated.

$$\text{Corrected mortality} = \frac{(\%\ \text{observed mortality} - \%\ \text{control mortality})}{(100 - \%\ \text{control mortality})} \times 100$$

The susceptibility or resistance status in mosquitoes will be reported based on the WHO criteria. The mortality between 98-100% indicates susceptibility (S), mortality between 90-97% indicated possible resistance (PR) and mortality below 90% indicates resistance (R) (WHO, 2016). Mortality of less than 98% is suggestive of the existence of resistance and further investigation is needed. If the observed mortality (corrected, if necessary) is between 90% and 97%, the presence of resistant genes in the vector population must be confirmed by additional tests with the same insecticide on the same population or the progeny of any surviving mosquitoes (reared under insectary conditions), or by molecular assays for known resistance mechanisms. If at least two additional tests consistently show mortality below 98%, then resistance is confirmed. If mortality is less than 90%, confirmation of the existence of resistant genes in the test population with additional bioassays is not necessary, provided that at least 100 mosquitoes of each species were tested. The test with results less than 90% mortality is necessary for further resistance intensity tests with 5x and 10x in the same locality, The mono-oxygenase-mediated resistance mechanism and restoration of resistance with PBO-insecticide bioassay tests can also be conducted if PBO impregnated papers are available or ordered.

The CDC Bottle Assay:

The CDC bottle bioassay can be performed on vector populations collected from the field or on those reared in an insectary from larval field collections. Major advantages of bioassay are that different concentrations of an insecticide may be evaluated. However, it is suggested that if required to test five times (5X) or 10 times (10 X) of the concentration in a tube test, it can be got done with the support of selected international institutes, following WHO standard protocol.

Insecticide resistance management

Insecticide resistance management plan is based on the consideration that the plan will ensure supervision and monitoring of quality and effectiveness of coverage. The implementation of a resistance monitoring plan will depend on financial resources and its sustainability. The factors taken into consideration are ensuring capacity building, data collection, and interpretation of data on resistance. The crucial element considered in framing the IRM plan is that the data which will be interpreted will also be taken into account during decision-making process at the country level. The

IRM plan is in alignment with global guidelines, and the data generated during the implementation of IRM plan will be shared with WHO so that the WHO database is updated.

In accordance with GPRIM, it is suggested that for successful implementation of Insecticide resistance management plan, one inter-sectoral steering committee for vector control, at the national level, need to be constituted which will provide feedback to government about progress and recommendations on vector control for consideration. The public-private partnership during inter-sectoral coordination will facilitate the Department of Agriculture and Health to compile the data on use of insecticides or pesticides to assess insecticide pressure which is one of the main causes of precipitation of insecticidal resistance. The steering committee will review quarterly the vector control interventions and data generated on insecticide resistance so that the decision for next year is facilitated. The decisions taken for vector control to delay or avoid precipitation of insecticidal resistance should be shared with the regulatory body/authority.

> What should be done if insecticide resistance among mosquito vectors is reported with synthetic pyrethroid?
>
> - A recently published article highlighted the factors for effectiveness of ITN or LLINs. The paper reveals that insecticide resistance is only one part of the effectiveness of ITN or LLINs or the insecticides used for treatment of the nets.
> - If we focus only on insecticide resistance, we are missing the broader issues which are mainly on the coverage, usage, and maintenance.

The decision-making process of committee and the programme is very crucial to change the insecticide if resistance is confirmed in *An. culicifacies* or any malaria vector to pyrethroids, after susceptibility tests conducted as per WHO recommended procedure with diagnostic dosage and intensity dosages of pyrethroid.

The use of insecticide for Indoor Residual Spray (IRS) in the malaria control operations started with DDT. The basic principle of IRS is to reduce the density and longevity of vectors so that the vectors do not survive till the malaria parasite requires to complete its development in the mosquito and the vector mosquito turns from infected to infective. Usually, if DDT resistance is established, the insecticide of organophosphate group like malathion is used, and if resistance against organophosphate group is established synthetic pyrethroid is introduced. Carbamate insecticides are the options after synthetic pyrethroid. However, the epidemiological impact is very important for analysis before a decision on change of insecticide is taken. The recently published paper on the effectiveness of LLIN (indicated in the box), also reflects that even if insecticide resistance is reported following WHO procedures, the insecticide need not to be changed immediately, rather a comprehensive field survey and analysis should be undertaken to understand the actual coverage of the IRS or LLIN used as a tool to prevent mosquito bite and reduce its density to minimize the probability of human mosquito contact.

In India, the insecticide change policy under National Vector Borne Disease Control Programme (NVBDCP) indicates that along with insecticide resistance, the epidemiological impact is to be seen together. It elaborates further that if insecticide resistance is reported but the epidemiological impact is visible then there is no need to change the insecticide[1].

Tasks, Activities and Timelines

- The first task will be the monitoring of insecticide resistance in the endemic areas, followed by its analysis along with epidemiological data and finally making a decision on management of insecticide resistance, either by rotation of insecticide or mosaic spray, following WHO guidelines to delay the precipitation of resistance.
- Monitoring of Resistance- the first step will be the selection of sentinel sites (villages) for IRM. This will include the preliminary entomological and epidemiological survey to know malaria cases, breeding sites, and more congenial resting places for mosquitoes so that more mosquitoes can be collected. The broad selection at the Gewog level has been done in consultation with programme officials, as depicted in the flow chart earlier but village selection will have to be done as detailed in following the para.
- The next step will be to go to those villages, which are reporting more malaria cases, for collections. The collections will be made pre-monsoon, monsoon, and post-monsoon seasons to know which month will yield maximum mosquitoes. Based on those results, the month and the villages will be identified.

The Activity Plan

Based on the collection of mosquitoes, one village in each Gewogs (sub-district) will be selected in identified districts making a total of 8 sentinel sites.

- Mosquito collection will be made from these 8 sentinel sites. Tentatively, these 8 sentinel sites will be fixed for minimum three years for collection of adult mosquitoes to insecticide resistance.
- In cases where the required number of adult mosquitoes in the collections is adequate, the susceptibility test will be performed with field-caught mosquitoes. However, in case, a small

[1] https://nvbdcp.gov.in/Doc/Malaria-Operational-Manual-2009.pdf

number of adult vector species is collected, the mosquitoes will be kept for egg laying and then the F1 generation of adult vector species would be subjected for conducting the test.

- The team of entomologists and insect collectors will be sensitized with a checklist, duration of field visit and how to plan collection. One example has been cited; however, it should be adjusted within feasible resources. The option will be to go to field on Monday, stay, discuss with villagers, and move in the night to the field area to do whole night biting/landing collections.
- This will indicate the peak biting time of vectors. The indoor and outdoor resting collection will also be done next morning (6 am to 10 am). The next day, after finishing the collection, mosquitoes will be brought to the temporary lab in the field or the place where arrangements have been made.
- The full-fed mosquitoes should be allowed for 4-5 hours so that they become half-fed. The tests in the field can be done around 5 or 6 PM. The team will stay till next day 7 PM, record the data after 24 hours and then return to the place.
- Thus, the team must plan for Monday to Wednesday evening as working and then they can return either on Wednesday night or Thursday morning. The team should go prepared for three to four nights in the field. However, the local challenge, as detailed, need to be considered and resolved at local level. The example is shown in the box:

> - The districts or PHC do not have entomologist or trained technicians & lack capacity to do susceptibility tests. Therefore, 1 Central Programme Entomologist + 1 Technician + 3 Insect Collectors need to proceed from Gelephu, Sarpang district.
> - The sentinel sites within Sarpang district can be reached within 3-4 hours journey by car but need at least 4 days (5 nights) to do 2-3 sets of tests with one discriminating dosage.
> - The field cattle bait collection mosquitoes are always mixture of even 5-8 species during monsoon season and we hardly get target species number 100, even after three to four sets of bioassays.
> - Further, if resistance is indicated the team need to do additional 2-4 bioassays for resistance intensity with 5x, 10x and synergist/PBO-insecticide tests. So, minimum of 10 days or 15 days are required for complete set of bioassays with at least 1-2 mosquito species with recommended numbers exposed (above 100) numbers.
> - During windy or heavy raining at night, the collectors return empty handed even during monsoon season. From November till February, team do not collect enough samples due to cold winter which is the limiting factor for tests in winter.

Annual Work-Plan for National Insecticide Resistance Monitoring and Management Plan Implementation

Type of sites for IRM

- IRM zone: A **zone** comprising geographically contiguous districts.
- IRM Unit: The identified **District** for IRM.
- IRM implementation Unit: **Gewog** (Block or Sub-district) within the IRM district comprising few Chiwogs (PHCs) and villages.
- IRM sentinel site: **Village** within the IRM Implementation unit.

Selection of sites

1. **IRM Zone:** In case of many districts, a zone is considered which ideally covers **3-4** geographically contiguous districts so that, the topography of the testing sites is identical to each other. In Bhutan, the number of malaria-endemic districts is 8. These 8 districts will be covered by conducting susceptibility tests in 5 districts so these 5 districts will be considered as 5 zones. Usually, the group of districts are covered in one zone or an individual district is made as zone depending on resource availability.
2. **IRM unit**: The criteria for selection of unit/district within each IRM zone will be endemicity (past or current), vector density and IRS exposure. Five districts namely Sarpang, Samdrupjongkhar, Samtse, Dagana and Zhemgang district have been identified in consultation with programme officials.
3. **IRM implementation unit**: Implementation units are the four gewogs in Sarpang district namely Sershong, Gelephu, Dekiling & Senggey Gewogs. Pemathang Gewog of Samdrup jongkhar district, Norbugang Gewog of Samtse district, Lhamoizingkha Gewog of Dagana district and Pangbang Gewog of Zhemgang district.
4. **IRM sentinel site:** Village within the IRM Implementation unit are the sentinel sites where regular IRM will be done. One village in each identified Gewog will be selected and fixed as a sentinel site.

Period of study

Prevalence of vector may be perennial, seasonal, or restricted to few months in a year in different districts. Based on such available data, period of susceptibility study may be fixed for the district which will be once a year, in the month when an abundance of vector mosquitoes is maximum. In Bhutan, it will be between June to October.

Human resource to be deployed for IRM by Tube test

The districts or PHC do not have Entomologist or trained technicians and lack capacity to do susceptibility tests. Therefore, 1 Central Programme Entomologist + 1 Technician + 3 Insect Collectors need to proceed from Gelephu, Sarpang district.

Sl. No.	Category of Human Resource	No. required
1	Entomologist	1
2	Technician	1
3	Insect Collector	3
4	Driver	1
	Total	**6**

Number of studies in a calendar year

Considering the average man-days required for a zone to complete the susceptibility study, it is estimated that one round of susceptibility study can be taken up in each IRM Unit in a calendar year.

Protocol for susceptibility study

The standard protocol of WHO as detailed in Second edition of "Test procedures for insecticide resistance monitoring in malaria vector mosquitoes-2016" shall be followed for carrying out susceptibility study by Tube test method to monitor insecticide resistance[2].

For bottle assay and to understand the mechanism of resistance, technical support of international institutions with separate financial help will be required if it is undertaken.

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[2] (https://apps.who.int/iris/bitstream/10665/250677/1/9789241511575-eng.pdf)

Financial assistance required (in Dollars)

Generally, the plan includes activities like designing a detailed plan for monitoring, capacity building of entomological teams, bioassay tests for susceptibility and molecular-biochemical testing for resistance mechanism. The indicative costs for IRM in 10 sentinel sites and 40 proposed sites are indicated below:

Activity	No. of Sentinel Sites	
	10	40
Planning Design	US$ 15000	US$ 15000
Capacity Building	US$ 33000	US$ 33000
Bioassay tests for susceptibility	US$ 50000	US$ 200000
Molecular tests	US$ 18700	US$ 74800
Total cost	US$ 116700	US$ 322800
Total cost per site	US$ 11670	US$ 8070

This is an indicative cost taken from "*Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM)*"[3]

The detailed financial plan for implementation, excluding support of WHO susceptibility test kits and insecticide-impregnated papers, has been developed and annexed.

Calendar of Activities

The entomological surveillance will be done on a monthly basis. The insecticide resistance monitoring will be done once a year. The activities will be done from June to October as detailed earlier in this section. The **Excel sheet Matrix** is attached indicating the monthly tentative schedule of activities including preparation, conduction, compilation, reporting, deliberation in committee, decision, and dissemination.

Annual Budget

The excel sheet with a tentative budget is attached. The total cost is tentatively around 52986 USD for three years, excluding the cost of salary of regular staff. This budget is based on 8 sites for IRM in 5 districts with the justification that malaria is very low, and it is expected that the density of vectors will also be very low so we may have to devote more days in field.

[3] Reference: http://whqlibdoc.who.int/publications/2012/9789241564472_eng.pdf

Suggested Reading

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2. Criteria and meaning of tests for determining the susceptibility or resistance in insects to insecticides. Geneva: World Health Organization; 1981.
3. Donnelly MJ, Isaacs AT, Weetman D. Identification, validation, and application of molecular diagnostics for insecticide resistance in malaria vectors. Trends Parasitol. 2016;32(3): 197–206.
4. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Geneva: World Health Organization; 1981.
5. Global plan for insecticide resistance management in malaria vectors. Geneva: World Health Organization; 2012 (http://www.who.int/malaria/publications/atoz/gpirm/, accessed March 2017).
6. Global report on insecticide resistance in malaria vectors. Geneva: World Health Organization; 2018 (http://www.who.int/malaria/publications/ atoz/9789241514057/en/, accessed 16 May 2018).
7. Guideline for evaluating insecticide resistance in arthropod vectors using the CDC bottle bioassay. Atlanta, GA: Centers for Disease Control and Prevention; 2010 (http://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_ en.pdf, accessed 15 September 2016).
8. Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. Atlanta: Centers for Disease Control and Prevention; 2011 (http://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ ir_cdc_bioassay_en.pdf, accessed March 2017).
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10. NVBDCP 2009: https://nvbdcp.gov.in/WriteReadData/l892s/Malaria-Operational-Manual-2009.pdf
11. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd ed. Geneva: World Health Organization; 2016 (http://www.who.int/malaria/publications/atoz/9789241511575/, accessed March 2017).

Budget Sheet for Implementation of IRM Plan

District	Name of Sentinel sites (Gewog)	IRM units district	IRM IUs (Gewog per District)	IRM sentinel sites (Villages 1 per Gewog/ district)	Total no. of site per district	Man-days required per Site	Man-days required per district @ 5 days per IU	Period of testing and entomological surveillance	Human resources required for every IRM Zone					Transportation charges			Fixed Honorarium (only for IRM)	Logistic support (Lumpsum of NU 3000 per IRM unit)	Total per year	Total for 3 years ((NU)	Total for 3 years (US Dollars)
									Entomologist	Technician	Insect collector	Driver	Total HR	Avg 100 km per study per IRM Unit	Amount required per district @ NU 4000 per trip	Amount required per district	For 6 members total per day@ NU 5300				
Sarpang	Sershong, Gelephu, Dekiling & Senggey Gewogs	1	4	4	4	6	120	Jul-Oct	1	1	3	1	6	100	4000	4000	636000	3000	643000	1929000	26068
Samdrup jongkhar	Pemathang Gewog	1	1	1	1	6	30	Aug	1	1	3	1	6	100	4000	4000	159000	3000	166000	498000	6730
Samtse	Norbugang Gewog	1	1	1	1	6	30	Sep	1	1	3	1	6	100	4000	4000	159000	3000	166000	498000	6730
Dagana	Lhamoizing kha Gewog	1	1	1	1	6	30	Oct	1	1	3	1	6	100	4000	4000	159000	3000	166000	498000	6730
Zhemgang	Pangbang Gewog	1	1	1	1	6	30	Oct	1	1	3	1	6	100	4000	4000	159000	3000	166000	498000	6730
TOTAL																			**1307000**	**3921000**	**52986**

Note: The cost of WHO Susceptibility papers and kits is not included as it is supplied by WHO

Honorarium to HR proposed to be involved in IRM

	Number	Honorarium rate per day per person	Honorarium Rate per day for total persons
Entomologist	1	1500	1500
Technician	1	1250	1250
Insect collector	3	3000	3000
Driver	1	1000	1000
TOTAL	**6**	**6750**	**6750**

Matrix for Calendar of Activities

District	Name of Sites (Gewog)	Preparatory Phase for Logistics and Training		Field Visits, Collection of Vector Mosquito and Test					Data Compilation and Reporting		Deliberation in Committee	Decision Approval	Dissemination of Decision
		Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Sarpang	Sershong Gewog												
Sarpang	Gelephu Gewog												
Sarpang	Dekiling Gewog												
Sarpang	Senggey Gewog												
Samdrup jongkhar	Pemathang Gewog												
Samtse	Norbugang Gewog												
Dagana	Lhamoizingkha Gewog												
Zhemgang	Pangbang Gewog												