Ethiopian Public Health Institute (EPHI)

Bacterial, Parasitic and Zoonotic Diseases Research Directorate

Public Health Entomology Research Team (PHERT)

Anopheles mosquito rearing and insectary handling guideline

March, 2017

Addis Ababa, Ethiopia

Ethiopian Public Health Institute (EPHI)

Bacterial, Parasitic and Zoonotic Diseases Research Directorate

Public Health Entomology Research Team (PHERT)

Anopheles mosquito rearing and insectary handling guideline

Authors

Araya Eukubay

Abate W/tensai

Getachew Eticha

Dr.Wendatir Nigatu

Dr.Adugna Wayessa

Wendimeneh Mekuriaw

Yonas Wuletaw

Alemnesh H/mariam

Fitsum Tesfaye

Tefera Asfaw

Esays Kinfe

January, 2017

Addis Ababa, Ethiopia

Table of contents

List of Figures

Acronym

EPHI	Ethiopian Public Health Institute
HEPA	High-Efficiency Particulate Air
IM	Intramuscular
IRS	Indoor Residual Spray
LLIN	Long-Lasting Insecticidal Net
PHERT	Public Health Entomology Research Team
NSP	National Malaria Strategic Plan
NMCP	National Malaria Control Program
RH	Relative humidity
SOP	Standard Operating Procedure
URL	Uniform Resources Locator
WHO	World Health Organization

Preface

Malaria is an important public health problem in Ethiopia. The country's started fight against malaria more than half century ago. The current stratification indicated that the proportion of population at risk of malaria was reduced to 60% from 68% of the previous stratification. According to National Malaria Strategic Plan (NSP) of 2014-2020 Ethiopia is planned to reduce malaria cases by 75% and eliminate malaria in selected low transmission areas by 2020.Vector control interventions are critical components of the strategic plan to meet the target. However, the emergence and spread of insecticide resistance is becoming the major challenges for the current intervention tools. The National Malaria Control Program (NMCP) and Ethiopian public health institute (EPHI) together with other partners selected and approved 25 sentinel sites from which information on susceptibility status of vectors and other entomological data can be collected. Ethiopian public health institute as a national research institute is responsible for evaluation of vector control tools: long-lasting insecticide nets (LLIN), indoor residual spraying (IRS) and susceptibility status of vectors to the recommended chemical insecticides. In order to successfully undertake those activities and contribute to the concern of fighting malaria, it's mandatory to have well established insectary and working guideline. Ethiopian Public Health Institute (EPHI) particularly Public Health Entomology Research team (PHERT) run an insectary for the last decade but has no institutionally endorsed insectary guideline yet which initiated us to write this guideline. Thus, we hope that this guideline will help personnel working in the insectary to rear reliable and high-quality *Anopheles* mosquitoes.

The guide line provides users with detail standard operational procedures of *Anopheles* mosquito rearing, and introduces way of designing and handling of insectary. It helps entomologist, insectary managers and entomology technicians to produce high quality *Anopheles* mosquitoes in the laboratory and field settings for different research and operational purposes.

This guideline has nine sections. Section one deals with introduction to malaria vectors and their distribution and insectary backgrounds. Section two concerns purpose and potential users, section three with insectary design and construction together with the facilities required. Section four deals with the microclimate required for an insectary. section five, six, seven, eight, and nine details on biology of *An.arabiensi*, *Anophele*s mosquito rearing procedures, insectary technician responsibilities, insectary cleanliness, safety and security measures respectively.

1. Introduction

Malaria remained the major public health problem worldwide. Globally, malaria case incidence and mortality rate decreased by 21% and 29% between 2010 and 2015 respectively (WHO, 2016). Currently the proportion of populations at risk of malaria in Ethiopia is reduced from 68% to 60% (FMoH 2014b). Malaria is transmitted with the bite of the vector female *Anopheles* mosquitoes. About 528 species of *Anopheles* mosquitoes have been described in the world, and approximately 80 of them are important vectors of malaria, filarial nematode and encephalitis virus (Manguin, 2013). *Anopheles gambiae* complex is the most important vector of malaria in sub-saharan Africa (Onyabe and Conn, 2001; Moralis, 2005). It is thought that Ethiopia comprises about 45 species of *Anopheles* mosquitoes of which *An.gambiae s.l* is the most prevalent (Ayele, 2016). *Anopheles arabiensis* is the main malaria vector in Ethiopia with *An.pharonsis*, *An.funestus* and *An.nili* considered as secondary vectors (Kenea, *et al*, Taye, *et al*, 2016). *An. arabiensis*, the species of dry, savannah environments or sparse woodland is considered as a zoophilic, exophagic and exophilic species (FMoH, 2014a). However, it has a wide range of feeding and resting patterns, depending on geographical location.

Scientists rear mosquitoes in the laboratory for different research purposes. Mosquitoes can be reared either in an insectary or directly in the field. An insectary is a place where mosquitoes can be reared under laboratory conditions (Williams, 2012). It may be a separate building, a room or section of room, usually modified or remodeled to suit the condition required for rearing (Gerberg, 1970). Mosquito insectaries vary widely in their sophistication and cost. The requirements for good mosquito culture are easily met and can be achieved by both simple (inexpensive) and complex (expensive) means (MR4, 2014).The goal of organized mosquito rearing is to provide reliable, affordable sources of high-quality mosquitoes for their many important purposes. The rearing of mosquitoes is complex and demanding for several reasons. Larvae are affected by temperature, density and available nutrition (Spitzen and Takken, 2005).The basic materials and methods of mosquito rearing are similar to those described many years ago (Benedict *et al*. ,2009) Personal experience and inherited methods rather than controlled experiments have been the mainstay for developing mosquito rearing, and these methods are sufficient for laboratory use.

Vector control is an essential component to mitigate mosquito born diseases. Malaria vector control in Ethiopia accounts about half a century. The two main vector control interventions are long-lasting insecticidal nets and indoor residual spraying. Chemical larvicidal and environmental managements are also important control parameters. However, the spread of insecticide resistance is becoming threats to the control interventions.

The federal ministry of health developed five year strategies of insecticide resistance monitoring and managements. The national malaria control program (NMCP) in collaboration with Ethiopian Public Health Institute (EPHI) and other partners also selected and approved 25 sentinel sites from which susceptibility status of insecticides and other entomological data's can be collected. Ethiopian Public Health Institute (EPHI) as national research institute has the mandate to evaluate the efficacy of vector control tools both for registration (shipment) and monitoring their effectiveness. To meet these targets it requires laboratory reared susceptible *Anopheles* mosquitoes, well designed insectary and working guideline. Thus, this guide lines will help national and sentinel site insectary technicians to rear high quality and abundant *Anopheles* mosquitoes in the laboratory and field settings.

2. Purpose of the Guideline and Potential users

2.1. Purpose of the guideline

The guideline provides a detail of standard operational procedures (SOP) for *Anopheles* mosquito rearing. It also serves as a basis for handling, designing and processing of insectaries.

2.2. Potential users

The guide line is intended for use by sentinel site insectary technicians, entomology researchers and any university students who conduct research on *Anopheles* mosquito larvicide test, adulticide test, repellant test, susceptibility test, bio cone test, etc.

3. Insectary design and Construction

3.1. Building and Room layout

Mosquito insectaries vary widely in their construction and costs; however there are several criteria that must be adhered to for successful mosquito colony establishment and maintenance.

- All insectaries should be designed to prevent arthropod escape.
- An insectary should have the minimum requirements such as rooms with constant temperature, a way of controlling lighting and humidity and adequate containment to prevent escape.
- A store/prep room is also important for storage of materials and routine maintenance of cages and other materials.
- Entry and exit routes should have double doors or other barriers in place to prevent the mosquitoes escaping.
- Windowless rooms with no external walls are preferable so as to control temperature and light. As far as possible each insect rearing room should have an ante-room which can serve as a buffer zone to prevent undue disturbance of temperature and humidity on opening the door. The ante-room also serves to limit the escape of adult mosquitoes.
- The walls of the room should be smooth and painted white or a very light color. The paint must not be insecticidal.

- Rearing room should have large sink with hot and cold running water. It is an advantage to have a separate room for cleaning of cages and rearing trays or other equipment (WHO, 2013).
- When there is a need to maintain two or more mosquito colonies, the rearing rooms should have one or more screened partitions dividing the area into separate units.
- Contamination of the individual colonies can be largely eliminated when the space permits a separate entrance into each (Annex1) though interconnecting; screened doors between the sub rooms are satisfactory (James, 2016).

3.2. Furniture

- Rust-proof metal, fiberglass or plastic furnishings are preferable.
- Shelving should be easily adjustable and stand-alone units should be equipped with wheels so that they can be easily moved to clean equipment – and beneath it.
- Movable tables, benches, chairs, storage cabinets and larval tray shelves are preferable because permanently installed furniture's are not suitable for new arrangements to meet changing conditions.

3.3. Equipment /supplies for mosquito rearing

Many types of rearing equipments and supplies are available to run an insectary (Annex2). Some of them are described as follows.

Cages

- Cages usually have a frame of wood or metal covered with netting of cotton, synthetic fabric, or aluminum.
- The gauge of mesh should be such that the holes are not more than 1.1 mm across to prevent small adults from squeezing through mesh larger than this.
- The front of the cage should have a fabric sleeve wide enough to allow the introduction of small bowls (12 cm diameter) for egg laying. As cotton readily rots, synthetic netting is generally preferable.
- The base and back of the cage can be of plastic laminate material; a solid base is preferable to one of netting as it must support bowls containing pupae or water for egg laying.

- A wide variety of rearing cages have been developed for adult mosquitoes. The most important to be considered is size in relation to number of mosquitoes caged, and particularly the area of available resting space per mosquito.
- *Anopheles* mosquitoes can be colonized in cages 30 x 30 x 30 cm.
- As density affects mating, feeding and longevity a vertical resting surface of $1.8cm^2$per mosquito is commonly used for many mosquitoes. The ease with which mosquitoes can mate is also affected by the size of the cage.

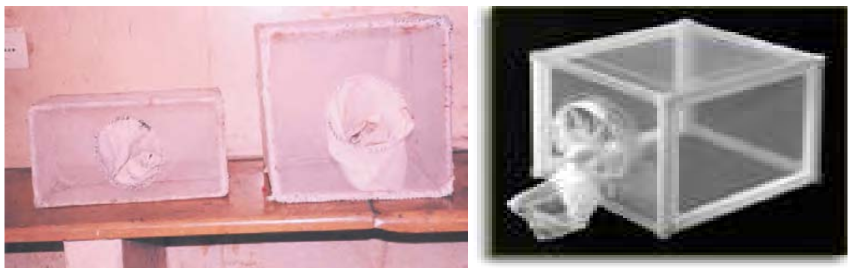


Figure1. Different design of mosquito cages

Aspirator

- The Mouth Aspirators (John W. Hock Company) (Models 412, 612, and 613) are used for aspirating mosquitoes in the lab and field.
- The Model 612 includes a 0.3-micron HEPA filter with screen to stop insect particles from entering the mouth.
- The Model 613 allows you to move insects from one cage to another without the possibility of escape.

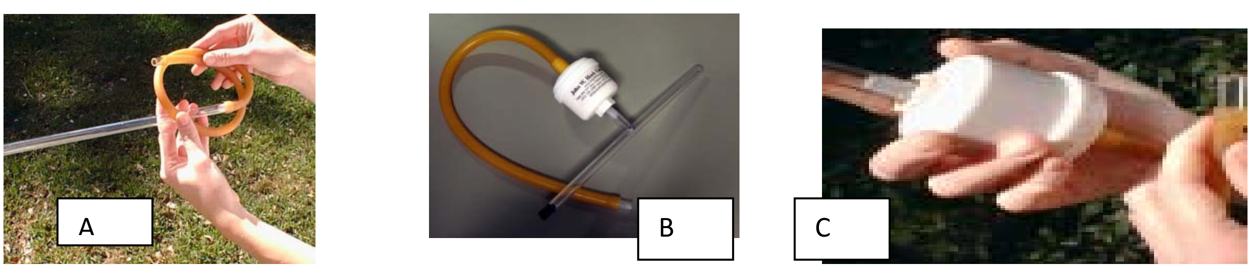


Figure 2a.Model 412 mouth aspirator **b**. Model 613Glunt two-way aspirator for mosquito with pathogen **c**. Model 612 mouth aspirator with HEPA filter

- **Larval Tray**
- Variety of containers capable of holding water can be used as rearing trays.
- For maximum holding capacities on benches or shelves rectangular shapes are better than round ones.
- White enamel trays made of polyethylene or polycarbonates are usually preferable for an insectary use.
- Trays about 30 x 25 x 5 cm are suitable for holding water to a depth of around 4 cm with 300 larvae per tray.

Figure 3. Different size of larval tray

Heater and Humidifier

Temperature and humidity can be adjusted using **Nikai** oil filled radiator and **Brune** product humidifier (see figure 8 and 9) respectively.

Figure 4a.Nikai Oil Radiator NOH838K (Heater) b. Brune-Product Humidifier

4. Microclimate required for insectary

Anopheles mosquitoes (Diptera, Culcidae) are among the successful groups of animals especially in conferring most of the ecological settings. *Anopheles* mosquitoes are found in all climatic zones of Ethiopia. However in laboratory conditions there is a need to adjust the micro climates such as temperature, humidity, and lighting and mimic the natural environments.

4.1. Temperature control

- Temperature control is a critical task in an insectary as it affects the life cycle of mosquito.
- Temperature control may be part of an elaborate air-conditioning system or a simple thermostatically controlled bar heater.
- If there is no fan-assisted air movement in the room, the temperature may be stratified between floor (coldest) and ceiling (warmest).
- Thermostatic controls are prone to fail after a relatively short period in a humid atmosphere and it is desirable to have a thermostat probe in the room with the switching device mounted externally.
- A modular system of readily replaceable thermostat and heater is an alternative.
- Regular temperature records should be made in the insectary.
- It is often convenient to have a maximum-minimum thermometer and/or recording thermo-hydrograph for this purpose.
- Insectaries used for mosquitoes are usually maintained within the range 25 °C–27 °C.

4.2. Humidity control

- Humidity regulation is mandatory as it play an important role in mosquito life span.
- Humidity control is maintained by a humidistat. As these have to be mounted in the room, the micro-switch eventually fails due to moisture and it is best to have both humidifier and humidistat replaced regularly.
- Humidity regulation can be also provided by small commercially available mist/aerosol producing devices. These have flat discs turned at high speed by an electric motor, resulting in a mist of fine water droplets being forced out through a top nozzle.

- Humidity should be measured regularly, either continuously with a recording thermo-hygrograph or daily using a whirling hygrometer.
- A range of 80±10% relative humidity is an often mentioned value to maintain adults.

4.3. Lighting control

- Both photoperiod and light intensity affect the development of various stages in the life-cycle, e.g. mating, feeding, egg-laying and time of pupation.
- Lighting periods are readily controlled by time clocks and switches built into the lighting supply.
- Standard daylight fluorescent lights are the most suitable source of lighting. Separate red light incandescent lamps may be provided for work during periods of darkness.
- A photoperiod of 12 hours of light and 12 hours of darkness is often preferable.
- Species which take the blood-meal at night can be kept in a room where the light cycle is offset. Light off at midday and light on at midnight allows routine work in the morning and blood- feeding of adult female mosquitoes in the afternoon.
- Some species may require a crepuscular period to swarm and mate, which may necessitate controls for automatic gentle dimming of lights to provide a dust effect and gradual increase in brightness to simulate dawn.

5. Behavior and Biology of *Anopheles* mosquito in the Laboratory

- Behavior and biology of *Anopheles* mosquito have a great role in making decisions in the insectary.
- The way the mosquitoes behave will affect choices of food, blood, egg laying and size of cages.
- Understanding of the differences between strains can be used to give clues of possible contamination.
- The mosquito life cycle is comprised of four stages: egg, larva, pupa, and adult. The egg, larval, and pupal stages are completed in aquatic habitats.
- The female mosquito oviposits 150 to 200 eggs on water surfaces.
- The location of these water surfaces ranges from natural habitats such as streams, vegetated ponds and rock-pools, to man-made habitats including wells and clay pots.

- Temporary water surfaces such as rain pools and hoof prints also serve as oviposition sites .The adult *Anopheles* female mates once and continue to lay eggs throughout its lifespan. Females must take a blood meal every 2-3 days. Blood is needed to develop eggs. Females will lay a batch of eggs before taking the next blood meal (Essop, 2014).

Egg stage

- The eggs of *Anopheles, Culex* and *Aedes*, are laid in different patterns and observing the patterns on egg collection can be one way of catching a cross-genus contamination event early (MR4, 2014).
- *Anopheline* eggs are laid singly and one to three batches of eggs can be laid during the average lifetime of a female mosquito.
- The eggs are able to float on the water surface with the aid of a pair of lateral, air-filled chambers.
- Hatching occurs two to three days post-oviposition, however environmental conditions can cause early and late hatching in certain egg batches.
- The length of time the eggs take to hatch into larvae largely depends on temperature: At about 30^{0}C, eggs hatch into larvae in about 2-3 days however, in temperate zones (16^{0}C), about 7-14 days.

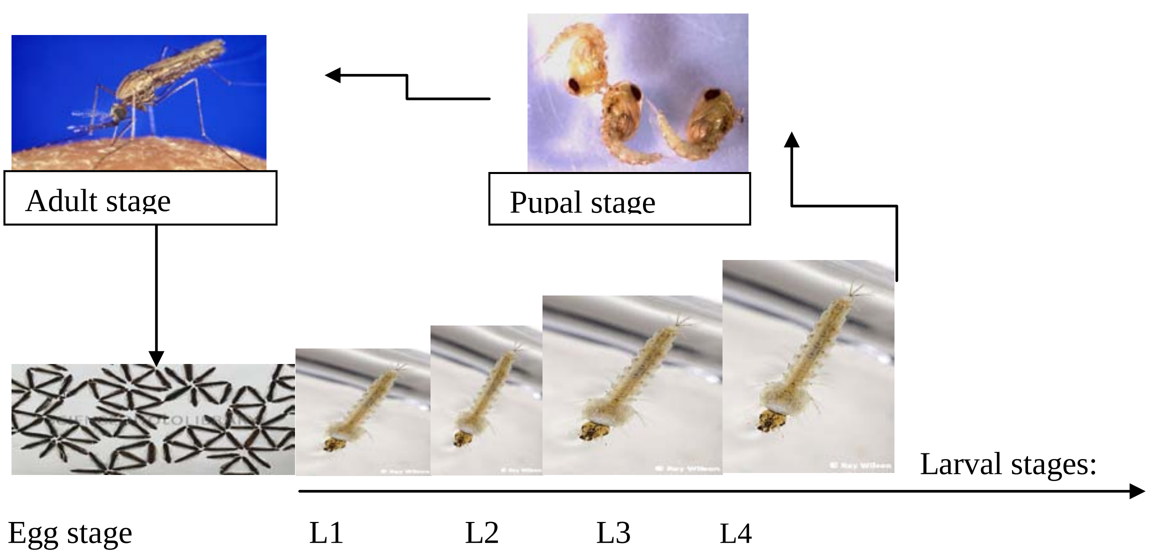


Figure 5.diagrame of the life cycle of *Anopheles arabiensis*

Larvae

- The *Anopheline* larva has no siphon and rests parallel to and immediately below the surface of water, whereas the *culicine* larva has a breathing tube (siphon) which it also uses to hang down from the water surface.
- Understanding of the method and location of feeding for the particular strain you are using helps in choosing a food.
- Many *Anopheles* and *Culex* use the feeding mode: **collecting filtering** which is feeding by removing particles that are suspended in the water column or at the water surface.

- Even though some *Culex* and *Anopheles* share the same method of feeding, the location of the feeding can be different. *Anophelines* tend to feed at the air/water interface or on the bottom while *Culex* and *Aedes* typically feed throughout the water column.

- Mosquito larvae have four stages (instars). The body size changes continually while the head capsule increases only at molts i.e. saltatorially.

Pupae

- Pupae of both *Anophelines* and *Culicines* are comma-shaped and hang just below the water surface. They swim when disturbed.
- The breathing trumpet of the *Anopheline* pupa is short and has a wide opening, whereas that of the C*ulicine* pupa is long and slender with a narrow opening. However, as it is difficult to distinguish *Anopheline* from *Culicine* pupae in the field, it is preferable to rear them in an insectary so that the emerging adult mosquitoes can be identified**.**

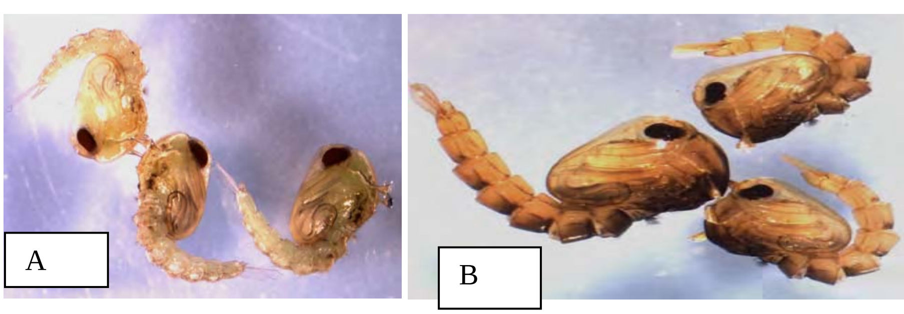


Figure 6a.*Anopheles gambiae* and b. *Aedes aegyptai* pupae

6. *Anopheles* mosquito rearing procedures

There are two means of starting colony in the insectary. These are either by collecting larvae or blood fed indoor resting females from the field. The basic steps are detailed as follow:-

- Collect larvae or blood fed indoor resting females from the field and put them in mosquito cages (for adults) and in the enamel trays (for larvae) in the insectary.
- Maintain them at the temperature of 25^0C-27^0C and 80±10% relative humidity (for adults).
- Blood feed adults raised from larvae in the insectary so as to lay eggs.
- Insert egg-lying petri-dishes containing little water and lined with filter paper into the adult cages.

Anopheles egg collection

There are several methods of egg collection. But in our laboratory, mosquitoes are allowed to lay eggs on wet filter papers supported with cotton and placed on petri-dishes.

Materials and chemical

- Petri-dish
- Cotton
- Filter paper
- Distilled water

Procedures

- Fill the base of petri-dish with distilled water and place cotton over it.
- Cover the surface with filter paper. Then
- Insert petri-dishes into mosquito cages and leave overnight.
- Take off petri-dishes from the cages the next day and remove dead mosquitoes with forceps.

6.4. Hatching *Anopheles* eggs

Anopheles eggs are usually hatched as soon as they are mature – normally about two days after they are laid. Once the eggs are hatched, they are given with diets consisting of small particles such as live baker's or brewer's yeast. The below protocol is prepared for eggs hatching.

Materials

- 2 % w/v active (live) baker's or brewer's yeast in purified water. Mix and use only that day.
- water wash bottle
- mosquito tray
- 5 ml pipette
- 50-100 ml bottle
- mosquito culture water

Procedure

1. Cover the bottom of the larval tray with distilled water.

2. Add 0.02% final concentration of yeast suspension (300ml water and 3ml of 2% w/v yeast solution) to each tray.

3. Swirl trays to distribute the yeast and put them on the shelf where they will be kept.

3. Hold the egging paper by the edge to avoid touching the eggs and gently rinse the eggs into the tray. Care must be taken not to splash eggs into an adjacent tray or onto the water bottle. Do not disturb or move the tray after adding the eggs as it can cause the eggs to stick to the sides of the tray above the water where they will dry and not hatch. If you accidentally jar the tray, gently rinse the eggs down from the side of the tray into the rearing water.

4. Make sure the trays are clearly labeled with strain name and date of hatching.

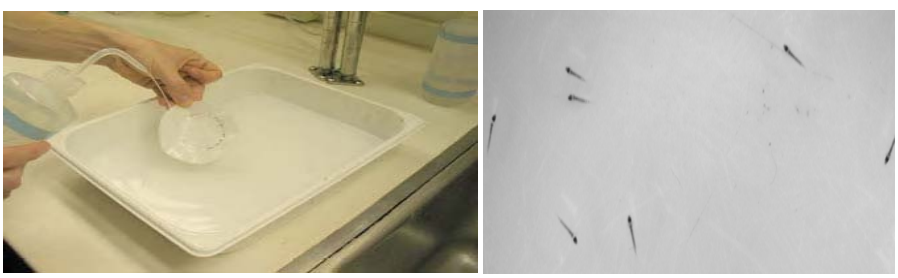
5. Cover the tray to prevent contamination that could occur from accidental splashing during egg rinsing into an adjacent tray and oviposition by loose females that can contaminate the stock.

6. *Anopheles* eggs will generally hatch immediately or within 24-48 hours after placement in water. On the day after placing the eggs in water, without disturbing the trays, gently uncover and scan to see if first instars larvae are present. If you do not see any 1st instars larvae, carry the tray carefully into a well-lit area and check again.

7. Allow one day between placing the eggs in water and splitting or thinning.

Add yeast slurry Tray with just enough water to cover the bottom

Hold egg paper in such a way that your finger do not touch the eggs



Gently rinse eggs in to tray
Cover and do not disturb for 24hrs

Check for larvae after 24hrs of rinsing

Figure7. *Anopheles* egg hatching diagram

6.5. Handling of Larvae

- Larvae are kept in trays or bowls. They are handled and transferred using glass pipettes fitted with rubber teats or bulbs.
- Larvae should be feed twice daily, usually with ground fish food. Both the quantity and quality of the diet are important to the longevity and fecundity of the adult stage. The first feeding should be 24hr after hatching. The first instars larvae require more food than the fourth instars.
- Larvae must always have ample food. However, care must be taken not to overfeed as this will lead to bacterial growth and death. Water should remain relatively clear and odor-free.
- Check trays in the morning and evening to ensure that there is food present and the need for thinning.
- If too much food is added to the larval tray there may be a problem with scum formation on the water surface causing the larvae to suffocate and die. Under feeding also produce smaller adults.
- Small quantities of grass, turf or other plants are sometimes added to the rearing water for *Anopheles* species.
- If larvae are overcrowded in trays, pupation is delayed and the resultant adults are smaller and weaker. This may have important consequences for experimental studies, e.g. such mosquitoes take smaller blood-meals.

6.6. Separating Larvae and pupae

A variety of mechanical devices have been described which can be used to separate pupae from larvae.

- Pupae must be removed from the larval trays shortly after they have formed otherwise the adults will emerge and escape into the rearing room.
- Pupae picking is usually carried out daily, the pupae being transferred to fresh water before transfer to adult holding cages.
- Pupae can be picked with teat-ended glass pipettes of the type used to handle larvae, or with fine net spatula.

- Large numbers of pupae may sometimes be separated from larvae by sieving the contents of a rearing tray and placing them into ice water. The larvae sink immediately while pupae float at the surface and can be skimmed off.

6.7. *Anopheles* adult caging and feeding

Adults are reared in different kinds of cages depending on the amount of mosquitoes needed to be reared, available materials for caging, security required and behavioral constraints of stocks. It is preferable to use large cages so as to promote mating and have enough resting space.

Anopheles adult feeding

- 10% w/v sucrose is common sugar sources for adult male and female mosquitoes.
- The sugar solution can be provided using soaked cotton balls lying on top of a cage if the mesh is non-absorbent (e.g. nylon rather than cotton).
- Cotton should stay wet enough for the mosquitoes to drink the sugar.
- Sugar pads should be changed every week because mold spores and fungi grow well on exposed sugar pads.
- 0.2% methyl-paraben added to a sugar source can extend the time before the sugar source begins to collect mold spores that are harmful to mosquitoes without causing early mortality or reduced longevity. In this case, sugar sources can be left unchanged for 30 days as long as they stay wet.

Feeding procedures

- Weigh out 50g of sugar per 500mL bottle.
- Add sucrose to clean bottle-the bottle should be in a good condition with an intact thread seal that is not chipped.
- Label bottle with date of preparation (date/month/year) and 10% Sucrose.
- Add 500mL distilled/deionized water and shake well to dissolve.
- Soak cotton with 10% sugar solution and put on the top of mosquito cages.
- Check sugar pads daily if they are wet to feed mosquitoes and for mold/fungus growth.

Female blood-feeding

- Egg production requires that females be fed to repletion on mammalian blood.
- Mammals are employed as a blood source because they provide a constant source of blood which is at the proper temperature, the animal provides necessary stimuli for feeding, and it is not necessary to handle blood directly.
- Anaesthetized mice, guinea pigs, rats, chickens, rabbit, or human volunteers arm can be used to feed mosquitoes. But in our insectary rabbits are used for mosquito blood feeding.
- Colonies are usually fed blood twice week.

Blood feeding procedures

- Shave rabbit on its flank using hair clipper or scissor.
- Inject it with 25-35mg/kg Ketamine: 5mg/kg Xylazine anesthesia intramuscularly (IM).
- Place it in the metal cage and tied properly.
- Put the rabbit on top of mosquito cage in such a way that the shaved part is placed towards the cage.
- Turn off the light and leave it for about an hour to feed mosquitoes.
- Provide food for guinea pig after it blood feeds the mosquitoes.

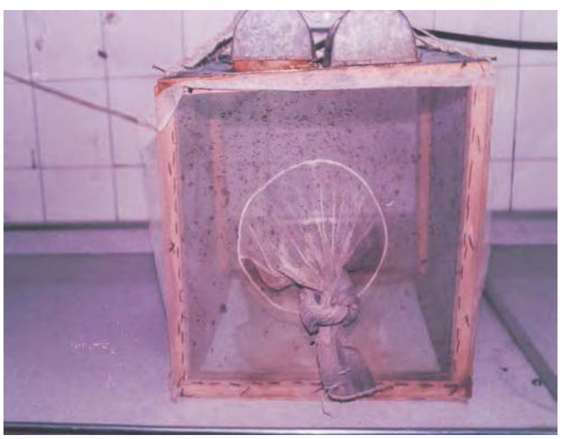


Figure8a.Blood feeding of Mosquito on rabbit b. Rabbit

Summarized Scheduling for *Anopheles* mosquito rearing

An efficient insectary requires fixed days for specific tasks such as egg collection and blood feeding. Having a strict schedule makes easier to share chores between technicians and produce abundant mosquitoes without risking the colonies. When researchers need *Anopheles* mosquitoes, they should pre-inform the insectary technician. Below is an example schedule for *An. gambiae* reared at constant 80%±10% RH and 27±2 ^{0} C.

Day1: Blood-feed adult females.

Day2: No attention required.

Day3: No attention required.

Day4: Insert egg dish to collect eggs.

Day5: Remove the egg dish and bleach the eggs.

Day6: Hatch larvae.

Day7: No attention required.

Day8: Feed and split/thin larvae.

Day9through Day11: Thin and feed pans as needed.

Day12 through Day13: Collect pupae or adults and feed larvae every day.

Day14: Blood-feed to reinitiate the cycle.

7. Insectary technician's responsibilities

1. Monitor of environmental conditions. Insectaries are constantly maintained at 25-27^{0}C. Relative humidity will be controlled to be in the range of 80% (± 10%) 365 days a year without interruption. Lighting is controlled in such a way that the total darkness between the end of sunset and the beginning of sunrise is 12 hours.

2. Larvae and adult feeding, collecting eggs, splitting of larvae, separating of pupa and maintaining of mosquito blood feed animals (rabbit …etc).

3. Perform Pest insect control continually to ensure essential absence primarily of ants and cockroaches. This is achieved in a way that no harm occurs to the insect colonies either directly or by contamination with toxicants transported by pests. Modifications of the facility are considered that physically reduce entry points, breeding sites, and harborages.

4. Control insect pests around the perimeter of the building to reduce external sources.

6. Operate and monitor mosquito traps or other killing devices continuously to prevent mosquito from escape.

7. Provide mosquitoes (egg, larvae, adult and pupa) for students conducting research up on request.

8. Preparing of adult mosquito for field upon request.

9. Installing and maintaining of air filtration properly to reduce the level of odors, fungi, dust, hair etc. Installing additional equipment or modifying existing equipment is considered to improve the air quality. Mold growing on mosquitoes and the insectary walls can be reduced by consistent attention to prevent spores.

10 Maintain the cleanliness and order of the storage areas.

11. Check up if mosquito food and blood sources are safe and of an adequate amount to ensure that shortages do not occur.

12. Proper packaging and reporting of mosquito samples collected from the field to the responsible personnel.

8. Insectary cleanliness

A clean environment is necessary for healthy mosquito culture and good research results. An inectary is a sensitive area which can be infected with pathogens such as bacteria, fungi and protozoan. These can be routinely transmitted via water or air. Primary infections may not be lethal but it may produce secondary infections which can be lethal. Thus reducing these pathogens is mandatory to maintain healthy insectary. There are different methods of cleaning and handling insectaries. The following are critical points that an insectary technician should consider for a good and aseptic maintaining of insectaries.

- Clean plastic containers, counter tops and floors with sodium hypochlorite (chlorine bleach) to reduce microbial infections.
- Keep unused larval and adult diets in refrigerator, as freezing kills many microbes.
- Never combine batches of old and newly prepared food.
- Replace the food container between batches or clean them with detergent soap and thoroughly dry in warm oven.
- Replace sugar pads regularly before they are grown molds.
- Store cotton balls in sealed containers.
- Discard dead mosquitoes from used containers as soon as possible.
- Keep dry wet pans, plastic containers and covers before use.
- Stack cups and trays in a way that promotes through drying.
- Keep mosquito cages clean and free of contamination.
- To make cleaning the walls as painless as possible, keep walls accessible for cleaning by using racks that can be easily moved or are on wheels.
- Wipe up spills and eliminate leaks to keep floors as dry and unfriendly for microbes as possible.
- Don’t let water accumulate on floors, containers or on counters.
- Remove unused equipment and supplies from insectary as they make it difficult to clean around and beneath.
- Keep shelves uncluttered, dusted and free of spills especially sugar water and food sources.
- Avoid cardboard; paper and wood instead use plastic, metals or glasses which can be easily sterilized with bleach or heat.

- Keep unused items sealed.
- Use drying ovens to sterilize plastic containers and other equipment that cannot withstand autoclaving.
- Routine cleaning of any water system should be done.
- Minimize pests in the insectary by reducing or eliminating the conditions that attract them such as food sources, harborages (shelter) and accessible water.
- Use Maxiforce Ant Bait Granules for outdoor ant trapping.

9. Safety and security measures

Special measures must be taken in the maintenance of colonies of insect vectors of diseases to isolate the insects from the surrounding environment and to prevent escape. In the case of malaria, mosquitoes escaping from a laboratory may seriously jeopardize control/elimination efforts (WHO, 2013). Escape of insecticide-resistant mosquitoes into a susceptible native mosquito population could have serious consequences for control measures in the area. The following control measures are applied for safe and secure insectary.

- Use double doors to limit mosquito escape from insectaries.
- Apply white painted walls/ante room for easily detection of escaped mosquitoes.
- Ante rooms should be installed with light-traps to further prevent escaped mosquitoes from reaching the outside.
- Place outdoor traps adjacent to insectary building to monitor and kill escapees and prevent entrance of wild mosquitoes from outside.
- Use of mosquito Magnet to trap escaped mosquito is also preferable.
- Traps should be monitored regularly and findings recorded.
- Screen drains from sinks to prevent the release of aquatic stages to the surrounding drains.
- Insectaries should not be sited in close proximity to animal rooms.
- When host animals are used to feed the mosquitoes, they should not be routinely housed in the same room to prevent infection with a vector-borne pathogen. Details of their care and procedures for feeding should be approved by the appropriate institutional or governmental oversight organisation.
- Access to the insectary should be restricted to people directly involved in research or support staff.

10. References

1. Ayele, A.A., 2016. Anopheles species and malaria transmission risk in a highland area , south-central Ethiopia.

2. Benedict, M.Q., Knols, B.J., Bossin, H.C.,Howell, P.I., Mialhe,E., Caceres, C., and Robinson, A.S., 2009. Colonisation and mass rearing : learning from others. *Malaria Journal*, 11, pp.1–11.

3. FMoH, 2014a. An Epidemiological Profile of Malaria in Ethiopia.

4. FMoH, 2014b. Disease Prevention and Control Directorate , Malaria Control Programme:National malaria strategic plan 2014-2020.

5. FMoH, 2016. Insecticide resistance monitoring and management strategy for malaria vector control in ethiopia.

6. Gerberg,E., 1970.Manual for mosquito rearing and experimental techniques.

7. James,B.,Kitzmiller and Done,W.,2016. Techniques for Rearing *Culex* Mosquitoes.The University of Notre Dame Micks sources:The American Midland Naturalist,Vol.52,No .1,pp.253-256.,52(1),pp.253-256.

8. Kenea, O.,Balkew, M., Tekie, H.,Gebreminchael, T.,Derssa, W.,Loha, E.,Lindtjorn, B. and Overgard, J.H., 2016. Human-biting activities of Anopheles species in south-central Ethiopia. *Parasites & Vectors*, pp.1–12.

9.

10. Essop,L., 2014. Fitness Assessments of Anopheles arabiensis Laboratory colonies from Southern Africa and their suitability for the Sterile Insect Technique.,pp.4-5.

11. Malaria Research and Reference Reagent Resource Center (MR4), 2014. Methods in Anopheles Research:forth edition., pp.1-90.

12. Manguin, S., 2013. Anopheles Mosquito - New insights into malaria vectors.

13. Moralis, I.and Frederic, S., 2005. Ppopulation structure of Anopheles arabiensis onlareunion island, Indian Ocean. , 73(6), pp.1077–1082.

14. Onyabe, D.Y. and Conn, J.E., 2001. The Distribution of Two Major Malaria Vectors, Anopheles gambiae and Anopheles arabiensis, in Nigeria. *Memorias do Instituto Oswaldo Cruz*, 96(8), pp.1081–1084.

15. Spitzen, J. and Takken, W., 2005. Malaria mosquito rearing – maintaining quality and quantity of laboratory-reared insects. , 16, pp.95–100.

16. Taye, B.,Lelisa, K., Emana, D., Asale, A.and Yewhalaw, D., 2016. Seasonal Dynamics, Longevity, and Biting Activity of Anopheline Mosquitoes in Southwestern Ethiopia. *Journal of Insect Science*, 16(1), p.6. Available at: http://jinsectscience.oxfordjournals.org/lookup/doi/10.1093/jisesa/iev150.

17. University of Colorado, Denver/Anschuz medical campus, 2012. Veterinary Anesthetic and Analgesic formulary; third edition., pp.16.

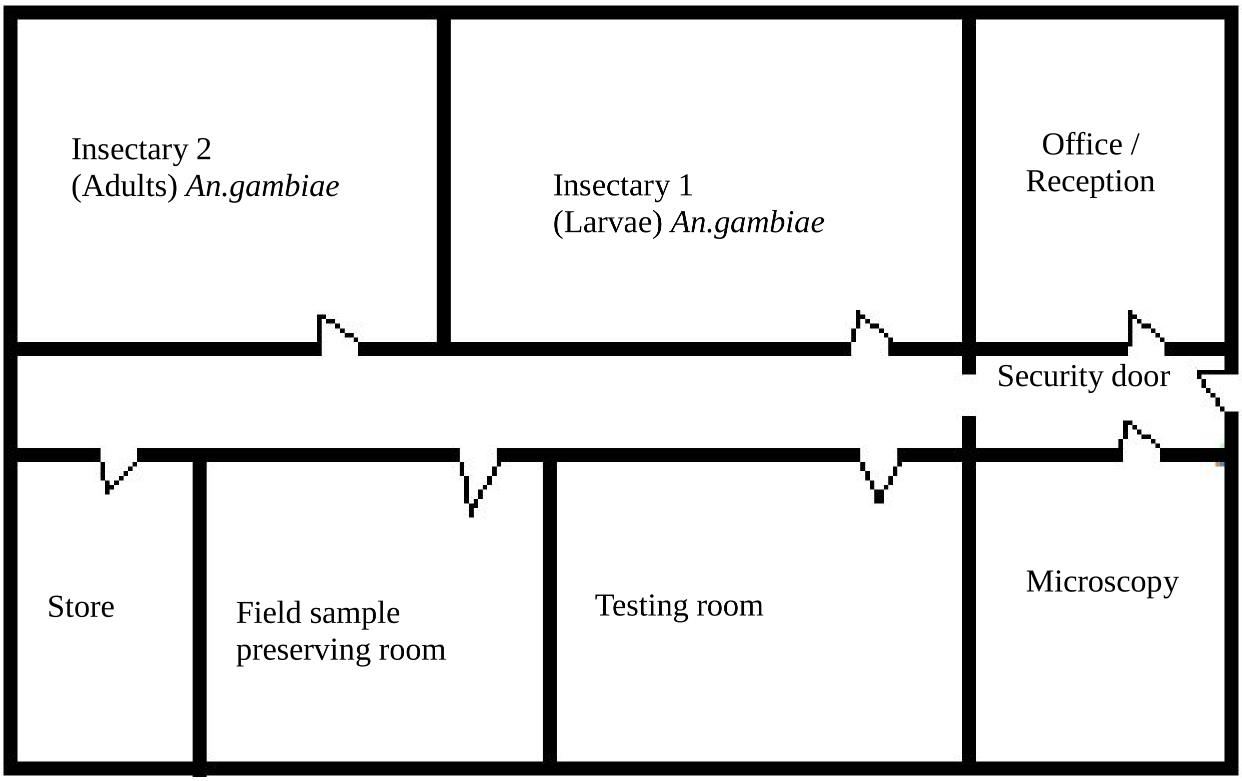
18.Vosshall Laboratory, 2014. Standard Operating Procedures for Rearing Aedes aegpyti Wild-Type colonies and individual strains. ,pp.1–30 available at. http://vosshall.rockefeller.edu/assets/file/Vosshall%20Lab%20Mosquito%20Rearing%20OP%20DEC%2012-2014.pdf

19.Williams, J., 2012. Training Manual on Malaria Entomology For Entomology and Vector Control Technicians.

20. World Health Organization (WHO), 2013. Malaria entomology and vector control , Participants Guide.

21. World Health Organization (WHO), 2016. world malaria report available athttp://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1.

Annex1. Insectary design



Annex2. Rearing supplies

Some useful supplies needed to equip an insectary are listed below on the table. Though some sources are local, the uniform resources locater (URLs) will provide information to give you an idea of what is described.

Mosquito Rearing Equipment/Supplies	Source	Model	URL
White plastic containers (for pupae)-approx. 250ml, used in food service	Fabricate locally		
12x12x12 Metal cage	BioQuip	1450B	www.bioquip.com
Adult cage large (Bug Dorm) 30x30x30cm	BioQuip	1452	www.bioquip.com
Adult cage small (collapsible) 20.3x20.3x20.3cm	BioQuip	145A	www.bioquip.com
Larval rearing trays	BioQuip	1426B	www.bioquip.com
Plexiglas covers for trays	Fabricate locally		
2 ml amber latex pipette bulbs	Fisher Scientific	S32324	www.fishersci.com
Plastic disposable pipettes (trim end, attach bulb and use as a pupae picker)	Fisher Scientific	13-711-7	www.fishersci.com
Stainless steel mesh strainer (to filter larvae and pupae)	Localy available		
Tubes for mixing yeast e.g. 15ml disposable	Fisher Scientific	05-538-51	www.fishersci.com
10 ml pipettes	Fisher Scientific	13-678-12E	www.fishersci.com
Sucrose (to make 10% sugar solution for adults)	Local source		

Large cotton balls	Fisher Scientific	07-886	www.fishersci.com
Colored tape (to label and discriminate stocks-choose 1 color per stock)	Fisher Scientific	15-901-15(color code)	www.fishersci.com
Larval diet e.g. Drs. Foster and Smith Koi Staple Diet	Drs. Foster and Smith	Koi Staple Diet	www.drsfostersmith.com
Stainless steel measuring spoons	Local source		
Mouth aspirator	John Hock Co.	412,612,613	www.johnwhockco.com
Feather-tip forceps	Bioquip	4748	www.bioquip.com
2 liter clear plastic pitchers with volumemarkings	Local source		
Filter paper sheets	Fisher Scientific	09-803-5E	www.fishersci.com
Qorpak tubes or similar	Qorpak	3891P	www.qorpak.com
500 ml wash bottles	Fisher Scientific	02-897-11	www.fishersci.com
Waterproof felt tip markers e.g. 'Sharpie'	Local source	13-379-1	http://new.fishersci.com
Indoor Natural Attractant Insect Trap			http://www.hammacher.com/Product/78397
Maxforce Ant Bait Granules			www.byerhome.co.za
Tetramin Fish food	Pet Mountain	16152	
Sugar feeder container	Fisher Scientific	02911944	www.fishersci.com
Plastic egg storage container Large	Amazon	7J76	
Plastic egg storage container Small	Amazon	B00GJ840DA	

Whirl Pack-Bag/Egg storage	Nasko	B01065WA	
Whatman filter paper circle	VWR	28450-048	
Porcelain Mortal	Fisher Scientific	S337621CR	www.fishersci.com
Porcelain Pestle	Fisher Scientific	S337621CRM	www.fishersci.com

Annex3. Registration book

An insectary needs a routine check up for temperature, relative humidity, egg, larva and pupa harvesting. The following format will be used for daily recordings in the insectary.

Date	Time	Temperature	Relative humidity	Batch No.	No. of Egg harvested	No. of Larvae collected	No. of pupa collected	No. of dead adults