

Protocol for mosquito rearing (*Anopheles gambiae*):

This protocol describes mosquito rearing in the insectary. The insectary rooms maintained 28°C temperature and ~80% humidity with 12 hrs day/night cycle. For this procedure you need, mosquito cages, 10% sterile sucrose solution, paper towels, beaker, whatman filter paper, glass feeders, human blood and serum, water bath, parafilm, distilled water, clean plastic trays, mosquito food (described below), mosquito net to cover the trays, vacuum and a collection chamber to collect adults.

Mosquito food:

Food A: grounded fish food (Aquaricare). A small pinch needs to be added.

Food B: grounded CAT food (Purina). A small pinch needs to be added.

Food C: cat food (Purina). Two tablets needs to be added.

Blood: Animal or human blood can be used to rear mosquitoes. Fresh blood is collected with a syringe and put in a sterile 15 ml Falcon tube containing 1ml of CPD (anticoagulant) for each 10 ml of blood, and is then mixed gently and centrifuged at room temperature at 2000 rpm for 5 min. The supernatant is discarded taking care to remove the buffy coat which comprises other blood cells (e.g. WBC). The RBC pellet is then suspended in an equal volume of RPMI medium by pipetting and further washed in this medium three times. After the final wash, the pelleted RBCs are resuspended in an equal volume of RPMI medium and stored at 4°C (can be kept for 8-10 days). Just before feeding, the RBC (in RPMI) is centrifuged (2000 rpm for 5 min) to pellet down the cells and the packed RBC is resuspended in serum (O+ human serum) to obtain a 40% haematocrit. The blood must be kept at 37°C always for the feeding.

DAY 1: The 3-5 day old adult female mosquitoes are fed on blood to lay eggs.

For blood feeding, an artificial membrane (parafilm) feeding method is used as follows: Red blood cells (see above for blood preparation) are mixed with heat inactivated serum to obtain a ~40% haematocrit (packed RBC 40% and serum 60%). This is then added to the glass feeder. The feeder is connected to a warm water-jacket (37°C) and placed on the cage to allow the mosquitoes access to the membrane surface. The mosquitoes are allowed to feed for ~30 minutes.

DAY 3: The females will lay eggs two days after they feed on blood. A small filter paper wrapped in a conical shape is put in a small beaker containing distilled water, making sure that filter paper gets moist. The beaker is kept inside the cage overnight, for the mosquitoes to lay eggs.

DAY 4: The filter paper containing the mosquito eggs is placed in a plastic tray with ~300 ml distilled water. A pinch of food A is added to the tray and eggs are allowed to hatch to larvae during the next days.

DAYS 5 - 8: Growing larvae are fed everyday with two tablets of food C and monitored for density and population. On the eighth day (5 day old larvae) the larvae population is

diluted from 1 tray to ~10 trays, with a pinch of food A and two tablets of food C in each tray. (~30 mins)

DAYS 9 - 12: The larvae are fed every day with food C and on the 12th day (9 day old larvae), the water is changed with fresh water and food is added (pinch of food B and two tablets of food C). The pupae starts developing at this stage and the trays are covered with nets to avoid escape of adults. (~ 8-10 mins)

DAY 13 - 15: The pupae are allowed to emerge to adults for the next 2 - 3 days. Food is given everyday to the larvae/pupae by carefully removing the net to avoid adult escapes.

DAY 16: The adults are collected into a cage through an aspirator connected to vacuum. The cage consists of a small 100 ml bottle with a cotton wick that is soaked with 10% sucrose (autoclaved) and a paper towel lining on the bottom to soak any potential sugar spills which may occur during cage handling. (~ 30 - 40 mins)

DAY 17-21: The adults (both male and females) are then kept in the insectary room for 4-5 days, fed on 10% sucrose before they are again blood fed to begin the next cycle. The same mosquitoes can be used to lay eggs more than once.

The mosquitoes that are not needed for experiments or rearing can be killed by placing the cage in a freezer. The used trays and cages need to be cleaned and dried before they can be used again.

NOTE: There can be some variations in the mosquito rearing method and different lab may have different techniques. Additional references on mosquito rearing are:

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6. Nasirian, H. and Ladonni, H. 2006. Artificial bloodfeeding of *Anopheles stephensi* on a membrane apparatus with human whole blood. J Am Mosq Control Assoc. 22:54-6.

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