Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

GUIDELINES FOR BIOSAFETY AND BIOSECURITY IN MOSQUITO REARING FACILITIES

Version 2.0

Food and Agriculture Organization of the United Nations

International Atomic Energy Agency

Vienna, 2023

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The proper citation for this document is:

FAO/IAEA. 2023. Guidelines for Biosafety and Biosecurity in Mosquito Rearing Facilities (Version 2.0), Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. Vienna, Austria. 7 pp.

GUIDELINES FOR BIOSAFETY AND BIOSECURITY IN MOSQUITO REARING FACILITIES

The present guidelines refer to a mosquito rearing facility which is suitable for work with uninfected mosquito vectors including: 1) mosquitoes that are already present in the local geographic region, and 2) exotic mosquitoes that upon escape would be inviable or become only temporarily established in areas not having active vector borne disease transmission.

The following are guidelines and actions, essentially equivalent to an ACL-2 level facility, to ensure (1) optimal health and safety conditions within facilities that contain mosquitoes capable of transmitting human pathogens and (2) biosecure operations.

A. Rearing Facility

Location of rearing areas or insectary. The rearing areas should be separated from areas that are used for general traffic of staff and offices within the building. The rearing areas should have no windows and each room should be temperature, humidity and photoperiod controlled based upon the requirements of the hosted insects. In case there is ventilation, or the air is regularly exchanged in the rearing rooms (normally 5 times/hr), the air ducts should be provided with appropriate high efficiency particulate air (HEPA) filters (mesh size 100 μm) to avoid escaped adult mosquitoes being sucked out of the building. All rearing facilities should be access controlled so that only authorised personnel can enter the rearing areas.

Insectary doors and air locks. Recommended entrance to the mosquito rearing areas is via a double-door vestibule that prevents flying mosquitoes to escape. The two contiguous doors cannot be opened simultaneously, and the sluice should be equipped with "insectecutors", mosquito traps and air-curtains. Door openings should in addition be covered with plastic biosecurity curtains / sheets or nets to minimize mosquito escapes and entrance of unwanted insects or other arthropods. Both doors must be self-closing.

Windows. Windows should not be present in the insect rearing areas, but only in the laboratory areas in the strict sense of the word (e.g. genetics or molecular biology). Windows must be resistant to breakage (e.g., double paned or wire-reinforced).

Pressurized rooms. Most insectary rooms should be under pressured to prevent escapes from the insectary to the corridors.

Interior surfaces. The insectary should be designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior walls are light-coloured so that a loose mosquito can be easily located, recaptured, or killed. Gloss finishes, ideally resistant to chemical disinfectants and fumigants such as epoxy paint, are recommended. Floors are light coloured, smooth and uncovered, but capable of heavy-duty loads. Ceilings are as low as possible to simplify detection and capture of flying insects.

Floor drains. Floor drains are equipped with appropriate filters (mesh size of 100 μm) to prevent accidental release of mosquitoes, especially mosquito eggs, larvae and pupae. In addition to appropriate filters, all wastewaters should be collected in collection tanks where the water can be heat- or acid-treated before being released in the mains.

Plumbing and electrical fixtures. Internal facility trimmings (e.g., light fixtures, pipes, ducting) should be kept to the absolute minimum (in number and size) since these provide hiding places for loose mosquitoes. Penetrations of walls, floors, and ceilings should be kept at a minimum and properly sealed.

Heating, ventilation, and air conditioning (HVAC) Ventilation and air exchange systems are recommended for mosquito maintenance but should not compromise containment of the insects.

Examples include: exhaust air is discharged to the outside without being recirculated to other rooms; appropriate filter/barriers (mesh size of 100 μm) are installed to prevent escape of adult mosquitoes; the insectaries where adults are maintained should be under-pressure (air will flow inward) to prevent escapes towards the corridors; fans located in the vestibule and internal corridors can be used to help prevent escape of flying adults; air curtains are a prerequisite and should be located at least in vestibules and doorways and optionally at the exit of each mass-rearing room.

Sink and shower. All sinks, showers and drains should have suitable filters and plumbing to prevent mosquito escape.

Facility compliance monitoring. The facility is evaluated regularly by the facility manager (and periodically by outside evaluators) for compliance to the necessary safety and security standards as outlined in this document. The facility manager ensures that alterations and maintenance have not compromised the containment characteristics of the building. Adequacy of the practices and facility in view of changes in research protocols, agents, or arthropods are considered.

B. Standard practices

Visitors – No short-term visitors are allowed in the mosquito area to prevent any potential bites by escaped mosquitoes. This to prevent escaped mosquitoes becoming infected by disease-carrying visitors, and that could later transmit the diseases to staff. Visitors can be shown the mosquito work in a display area (e.g. in the foyer of the building) and all mosquitoes on display should be sterilized. All long-term visitors (fellows, consultants, new staff) should present a medical certificate certifying that they are free of the major mosquito borne diseases before being allowed to work in the mosquito insectaries. If this certificate cannot be presented, they should be screened at a well-equipped biomedical facility for the presence of any asymptomatic infection with a known mosquito transmitted human pathogen before they are given access to the mosquito areas.

Location of mosquitoes. All equipment (cages, trays, incubators, etc.) containing mosquitoes are deployed in such a way that accidental contact and release is minimized. Therefore, the insects are located in specialised rooms, outside the flow of general traffic. Hallways should be avoided and only used when absolutely necessary (e.g., for cleaning purposes).

Supply storage. Equipment and supplies not required for operation of the insectary should not be located in the insectary. All supplies for insect maintenance that must be kept within the insectary should be located in a designated room and not on open shelves. It is recommended that a closed storage room, cabinets with tight-fitting doors or drawers be used. Doors and drawers are opened only for access. Insect blood diet should be kept in sealed containers at 5-7°C. Materials unrelated to mosquito rearing and experimentation (e.g., unused containers, clutter) that provide breeding sites should be minimized.

General insect elimination. Accidental sources of insects from within the insectary are eliminated. This may be accomplished by cleaning work surfaces after a spill of materials, including soil or water that might contain viable eggs. Mosquito traps should be located in all areas, including corridors. Pools of water should be mopped up immediately, and no containers with water other than those used for the rearing of larvae, should be left in and outside to the insectaries.

Larval tray – adult holding cage cleaning and disinfestation. Practices should be in place such that all stages of mosquito development including adults cannot escape by inadvertent disposal of trays and holding cages. Larval rearing trays should not be leaking to prevent egg, larval and pupae escaping. Larval trays should additionally be covered to prevent emerging adults from escaping. Larval trays and adult holding cages should be properly cleaned to prevent survival and escape (e.g., these can be heated to, or chilled below, the lethal temperature). They can also be disinfected chemically and/or autoclaved.

Adult holding cage. Cages used to hold adult mosquitoes have to be constructed in such a way that they effectively prevent escape. Screened mesh, if used, should be durable and of a size appropriate to prevent escape. Non-breakable cages are recommended. Cages should preferably be autoclavable or disposable. Openings designed to prevent escape during removal and introduction of mosquitoes are recommended.

Disposal of mosquitoes. Living mosquitoes are not to be disposed of. All waste from the insectary (including mosquito carcasses, and residual blood meals) are transported from the insectary in leak-proof, sealed containers for appropriate disposal in compliance with applicable institutional or local requirements. All stages of mosquitoes are killed before disposal, either through autoclaving, incineration of material, heat or cold treatment (insects can be killed with hot water/heat sterilizing oven or freezing before being disposed of).

Labelling. All colonies and individual cages should be identified adequately. Labels giving species, strain/origin, date of collection, generation number, responsible scientist or laboratory technician, etc. are firmly attached to the adult holding cages or the larval trays/racks.

Isolation of newly introduced mosquitoes. Spread of pathogens to uninfected mosquito colonies should be prevented. Colonies infected with pathogens should not be kept in the insectary and if detected, should be isolated in a separate room or destroyed. Establishing new colonies, either from material collected in nature or originating from existing colonies should be quarantined for a minimum of 2-3 generations upon arrival in the facility. The insects should be checked for mosquito transmitted human virus or mosquito pathogenic virus infections, and once their clean status is confirmed, they can be allowed in the general rearing areas.

Screening for diseases. All colonies should be screened periodically (e.g. each 6 months) for the presence of mosquito transmitted human pathogens (such as Zika virus, West Nile virus, filariasis, dengue fever, chikungunya, yellow fever and malaria) using PCR and RT-PCR.

Prevention of accidental dispersal on persons or via sewer. Personnel should take appropriate precautions to prevent transport or dissemination of mosquitoes from the insectary on their persons or via the sewer. Before leaving the insectary and after handling mosquito cultures, staff should wash their hands, taking care not to disperse viable life stages into the drainage system. Staff should always wear protective clothing that is left in the dressing areas and that can only leave the building for cleaning. Staff should preferably shower before leaving the insectary.

Pest exclusion. Entrance of unwanted wild arthropods (e.g., ants, psocids, houseflies, cockroaches, spiders) should be avoided at all cost, as this precludes predation, contamination, and possible inadvertent infection.

Monitoring of escaped mosquitoes. Facility managers should regularly assess whether escapes are occurring, and if yes, the extent. An effective mosquito trapping programme is recommended to assess the level of escapees. Adult traps (CDC traps, Bioagent Sentinel traps or ovi-sticky traps) for mosquitoes are recommended. Particularly in the case when exotic mosquitoes are reared, careful monitoring is recommended, also outside of the facility. Records of exterior captures should be maintained.

Breeding reduction. Mosquito breeding areas should be reduced as much as possible and detected breeding sites immediately eliminated. Furniture and racks should be minimized and should be easily moved to permit cleaning and location of escaped insects. Equipment in which water is stored or might accumulate (e.g., humidifiers) should be screened regularly to prevent mosquito access, or the water mixed with chemicals to prevent mosquito survival.

Routine decontamination. Equipment and work surfaces in the insectary are routinely decontaminated with an effective chemical or by radiation (e.g., heat) after actual or potential contact with an infectious agent, and especially after overt spills and splashes of viable materials (including water that might contain eggs).

Procedure and safety manual. All procedures should be carefully designed and implemented to prevent escapes and accidents. A safety manual should be prepared and rigorously implemented. The manual should contain emergency procedures, standard operating procedures, waste disposal and other information necessary to inform personnel of the methods for safe maintenance and operation of the insectary.

Training. Laboratory personnel are advised of special hazards and are required to follow instructions on practices and procedures contained in the safety manual. Adherence to established safety procedures and policies is made a condition of employment and is part of the annual performance review of every employee. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.

Medical surveillance. An appropriate medical surveillance programme is in place. Any evidence of allergies or reactions to the presence/bites of mosquitoes should be immediately reported to the medical authorities. In general, persons who may be at increased risk (e.g. during pregnancy) should not be allowed in the insectary and assigned tasks outside of the insectary.

Access restrictions. Routine access is limited to technical and professional staff. Cleaning and maintenance persons are made aware of the hazards present and the consequences of mosquito release and contact with escapees.

C. Special practices

Housing of non-arthropod animals. Animals not necessary for culture of the mosquitoes are not permitted in the insectaries. Animals used as hosts or blood sources or for special experiments (e.g. predators) may be housed in special rooms within the insectary but should be adequately protected from access by escaped mosquitoes. Under no circumstances it is allowed for personnel (staff, visiting scientists, fellows, interns, etc.) working in the insectary to feed mosquitoes *in vivo* on hands or arms.

Containment during blood-feeding. Mosquitoes fed on host animals are prevented from accidental transfer to the holding areas of the hosts. When handling/removing animals after exposure to mosquitoes, precautions must be taken to prevent adult escapes through screens, covers, and by flying. Host animals are inspected closely (e.g., concealment in fur, ears, crevices), and the cages should be sufficiently robust to prevent escape during feeding. Holding and using live animals for blood-feeding requires particular certifications and ethical permits depending on the regulations in the country. Blood-feeding using artificial systems with membranes should be preferred since it reduces risks and animal suffering.

Blood source. The blood source is considered as a source of inadvertent infection and transmission. Measures are implemented to prevent such an event. Use of sterile blood or blood from sources known to be pathogen-free is recommended. In contrast, use of blood from animals or humans whose infection status is uncertain is to be avoided. Common practice is to irradiate the blood with 1.5 kGy if an irradiator is available.

Handling of escapees. Escaped mosquitoes are killed or collected and properly disposed of. They are never returned to the colonies.

Accidental release reporting. The insectary director is notified promptly of accidental release of mosquitoes. All measures should be taken to kill the escapees promptly.

Detection of human arboviral pathogens. Any new field collected mosquitoes, colony or strain should be screened for the presence of arboviruses, such as chikungunya, dengue-2, Usutu, West Nile and Zika, before their transfer in the mosquito rearing facility. The detection can be done using a one-step real-time reverse transcription quantitative PCR (RT-qPCR) protocol as described in Hien et al., 2022 and Tang et al., 2020. This protocol is quite sensitive being able to detect a single infected individual in a pool of 1600 mosquitoes.

D. Safety equipment

Clothes. Gloves are worn when handling host animals or blood used to feed the mosquitoes. Laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood and vertebrate animals.

Arthropod-specific personal protective equipment. Personal protective equipment is worn as appropriate e.g., respirators for arthropod-associated allergies, particle masks, or head, covers. All personnel working in the mosquito area need to wear protective clothing (e.g. shirts with long sleeves (no T shirts), long trousers (no shorts), no sandals without socks, etc.) at all times. Aspirators for mosquitoes should include appropriate filters to prevent inhalation of mosquito scales, or automatic aspirators should be used.

References

Hien, A.S., Sangaré, I., Ouattara, E.L.P., Sawadogo, S.P., Soma, D.D., Maiga, H., Diabaté, A., Bonnet, E., Ridde, V., Fournet, F., Hawkes, F.M., Kaupra, C., Bouyer, J., Abd-Alla, A.M.M., Dabiré, R.K., 2022. Chikungunya (Togaviridae) and dengue 2 (Flaviviridae) viruses detected from Aedes aegypti mosquitoes in Burkina Faso by qRT-PCR technique: Preliminary results and perspective for molecular characterization of arbovirus circulation in vector populations. Front. Trop. Dis. 3, 920224. https://doi.org/10.3389/fitd.2022.920224

Tang, Z., Yamada, H., Kraupa, C., Canic, S., Busquets, N., Talavera, S., Jiolle, D., Vreysen, M.J.B., Bouyer, J., Abd-Alla, A.M.M., 2020. High sensitivity of one-step real-time reverse transcription quantitative PCR to detect low virus titers in large mosquito pools. Parasit. Vectors 13. https://doi.org/10.1186/s13071-020-04327-4