AUDA-NEPAD
AFRICAN UNION DEVELOPMENT AGENCY

Guidelines for Containment Facilities for Testing of Genetically Modified Mosquitoes

West Africa Integrated Vector Management Programme

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About The AU, AUDA-NEPAD and WAHO

The African Union (AU)

The African Union (AU) is a body of 55 member states that make up the countries of the African Continent. It was officially launched in 2002 as a successor to the Organization of African Unity (OAU), which ran from 1963 to1999. The decision to re-launch Africa's pan-African organisation was the outcome of a consensus by African leaders that in order to realise Africa's potential, there was a need to re-focus attention from the fight for decolonisation and ridding the continent of apartheid hitherto pursued under the OAU, towards increased cooperation and integration of African states to drive Africa's growth and economic development. The AU is guided by its vision of *An integrated, prosperous and peaceful Africa, driven by its own citizens and representing a dynamic force in the global arena [1].*

To realise this vision, the Africa Union developed and adopted a 50-year strategic plan called Agenda 2063 [2]. Agenda 2063 is the continent's strategic framework that aims to deliver on its goal for inclusive and sustainable development and is a concrete manifestation of the pan-African drive for unity, self-determination, freedom, progress and collective prosperity pursued under Pan-Africanism and African Renaissance.

The AU has been steadfast in proposing more enabling and science-based approaches to the challenges of the continent. Its report on gene drives clearly embraces the technology as a realistic option for effective disease control. A constructive development along this path was witnessed at the 29th Ordinary Session of Heads of State and Government of the African Union in Addis Ababa, where pursuant to Decision *Assembly/AU/Dec.649 (XXIX),* the session embraced the gene drive technology as a realistic option for malaria control. The session, in its decision, requested the African Union Commission (AUC), West African Health Organization (WAHO) and African Union Development Agency-New Partnership for Africa's Development (AUDA-NEPAD) to collectively support the initiative [3].

In 2018, through recommendations of the African ministers responsible for science and technology *EX.CL/Dec. 987(XXXII),* the Executive Council of the African Union encouraged member states to harness emerging technologies, including gene drive, in their development initiatives [4].

The decisions above have offered solid policy statements for the continent regarding gene drives for human health purposes, which have impacted discussions in AU member states. It is a basis for a harmonised approach for Africa in the development of policy regulations and guidelines such as this to facilitate the responsible and safe application of the technologies for research and subsequent deployment.

The African Union Development Agency - NEPAD (AUDA-NEPAD)

At the 31st Ordinary Session of the Assembly of African Union Heads of State and Government held in Nouakchott, Mauritania from 25th June to 2nd July 2018, the Heads of State and Government approved the transformation of the New Partnership for Africa's Development (NEPAD) Planning and Coordinating Agency into the African Union Development Agency (AUDA) as the technical body of the African Union with its own legal identity, defined by its own statute [6]. The objectives of AUDA-NEPAD are to: a) coordinate and execute priority regional and continental projects to promote regional integration towards the accelerated realisation of Agenda 2063; b) strengthen capacity of African Union Member States and regional bodies; c) advance knowledge-based advisory support; d) undertake the full range of resource mobilisation; and e) serve as the continent's technical interface with all Africa's development stakeholders and development partners.

The West African Health Organization (WAHO)

The West African Health Organization (WAHO) was established in 1987 when the Heads of State and Government from all fifteen countries in the Economic Community of West African States (ECOWAS) adopted and thereafter ratified the protocol for its creation. WAHO has transcended linguistic borders and hurdles in the sub-region to serve all fifteen ECOWAS Member States. The protocol grants WAHO the status of a specialised agency of ECOWAS and, as guided by its mission statement, 'the attainment of the highest possible standard and protection."

The regional agency is charged with the responsibility of safeguarding the health of the peoples in the sub-region through initiation and harmonisation of relevant policies of Member States, pooling of resources, and in cooperation with one another, maintaining a collective and strategic focus on important health problems of the sub-region.

WAHO has, through its strategic programmes, undertaken measures to combat malaria, malnutrition, HIV/AIDS as well as maternal and infant mortality. It has also spearheaded the prevention of blindness, increased access to medicines and vaccines, epidemiological surveillance as well as training and health information management in the sub-region.

Through its second strategic plan, WAHO is currently implementing various cutting-edge programmes in the sub-region to improve the overall health systems, ensure high-quality health services, develop sustainable financing of health and support institutional development within WAHO itself.

Acknowledgements

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Abbreviations

APET	African Union High Level Panel on Emerging Technologies
AU	African Union
AUDA-NEPAD	African Union Development Agency The New Partnership for Africa's Development Agency
BSL	Biosafety Levels
CRISPR/Cas9	Clustered Regularly-Interspaced Short Palindromic Repeats/ CRISPR-associated protein 9
DNA	Deoxyribonucleic Acids
ECOWAS	Economic Community of West African States
GD	Gene Drive
GMM	Genetically Modified mosquitoes
GDM	Gene Drive mosquitoes
GDMBSL	Gene Drive mosquitoes Biosafety Level
GDMCL	Gene Drive mosquitoes Containment Level
HEGs	Homing Endonuclease Genes
IBC	Institutional Biosafety Committee
IVM	Integrated Vector Management
OAU	Organization of African Unity
PI	Principal Investigator
RA	Risk assessment
RNA	Ribonucleic Acid
SOPs	Standard Operating Procedures
USDA	U.S. Department of Agriculture
VBDs	Vector-borne diseases
WAHO	West African Health Organisation
WA-IVM	West Africa Integrated Vector Management Programme
WHO	World Health Organization

Foreword

More than 80% of the world's population is at risk of one or more vector-borne diseases (VBDs), which together are responsible for 17% of the global burden of disease [1]. The AU is guided by its vision of An integrated, prosperous and peaceful Africa, driven by its own citizens and representing a dynamic force in the global arena. The AU has been steadfast in proposing more enabling and science-based approaches to the challenges of the continent. Its report on gene drives clearly embraces the technology as a realistic option for effective disease control.

A constructive development along this path was witnessed at the 29th Ordinary Session of Heads of State and Government of the African Union in Addis Ababa, where pursuant to Decision Assembly/AU/Dec.649 (XXIX), the session embraced the gene drive technology as a realistic option for malaria control. The session, in its decision, requested the African Union Commission (AUC), West African Health Organization (WAHO) and African Union Development Agency-New Partnership for Africa's Development (AUDA-NEPAD) to collectively support the initiative [3].

In 2018, through recommendations of the African ministers responsible for science and technology EX.CL/Dec. 987(XXXII), the Executive Council of the African Union encouraged member states to harness emerging technologies, including gene drive, in their development initiatives [4].

The decisions above have offered solid policy statements for the continent regarding gene drives for human health purposes, which have impacted discussions in AU member states, and ECOWAS is leading in the implementation of the policy. It is a basis for a harmonised approach for Africa in the development of policy regulations and guidelines such as this to facilitate the responsible and safe application of the technologies for research and subsequent deployment on the continent.

Considering the significance of these diseases, the Economic Commission of West African States (ECOWAS) has agreed on the establishment of a West Africa- Integrated Vector Management (WA-IVM) Programme. The purpose of this programme is to establish and operationalise a platform for the region to build strong collaborations among member countries on issues relevant to effective control of the vectors. Some of the key elements being considered include biosafety, environment, ethics, and regulatory oversight and health systems. The WA-IVM platform also aims to equip and capacitate the region with innovative technologies and new approaches for controlling the mosquito vectors. Considering that malaria is the most important vector-borne disease in sub-Saharan Africa, the WA-IVM Programme will use malaria as a pathfinder disease for developing its platform activities. This specific guideline is focused on mosquitoes that transmit malaria.

The advent of gene drives has brought new hope and opportunities for accelerating efforts towards malaria eradication. Gene drives can stimulate biased inheritance of specific genes to suppress or alter populations of select organisms, including mosquitoes. Laboratory studies have demonstrated that the technique could be applied to prevent mosquitoes from transmitting the malaria parasite or to significantly suppress the mosquito populations (2, 3). In addition, mathematical simulations have shown that other than certain concerns such as the potential rise of drive-resistant alleles, the desired genetic constructs could be rapidly driven through large mosquito populations across landscapes, thereby contributing significantly to sustainable malaria control and eradication, especially if used alongside current interventions (4). Such an approach would require lower levels of human resources, marginal logistical challenges and lower costs, thus potentially being more cost-effective even in locations currently considered remote.

To ensure the appropriate review and oversight of this new technology in West Africa, the AUDA-NEPAD Agency, in collaboration with ECOWAS, have initiated the development of guidelines to cover the different components necessary to safely use the genetically modified mosquitoes technology.

This specific guideline focuses on containment facilities and containment processes relevant to genetically modified mosquitoes.

Glossary[1]

Alleles – two or more alternative forms of a gene that arise by mutation and are found at the same location on a chromosome.

Community engagement (also referred to as public engagement) – the process of working collaboratively with and through groups of people affiliated by geographic proximity, special interest, or similar situations to address issues.

Containment - the action of keeping something under control or within limits. Contained use could be defined as any operation undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment.

Deployment – implementation of GDM technology as part of a national or regional programme for vector control.

Gene drive – a mechanism that increases the transmission of a transgene in a population, above that which would be expected based on Mendelian inheritance. The increase is reflected in the excess proportion of progeny that carries the transgene.

Ecosystem – a biological system composed of a community of organisms and the non-living environment with which it interacts.

Endemic – a situation in which disease is present continuously at some level in an area.

Endpoint – an event or outcome that can be measured objectively to determine whether the intervention being studied has the desired effect.

Ethics – an activity or inquiry intended to shed light on the correctness or justifiability of a given course of conduct.

Ethics committee (institutional or national ethics committee, institutional review board or ethical review board) – a group charged with providing oversight for biomedical and behavioural research involving humans and animals, with the aim to protect the rights and welfare of research subjects.

Fitness – description of the ability to both survive and reproduce and is equal to the long-term average contribution to the gene pool by individuals having a particular genotype or phenotype. If differences between alleles of a given gene influence the fitness of an organism, then the frequencies of these alleles will change over generations, and the alleles with higher fitness will become more common than the weaker alleles that are less adapted for survival and reproduction.

Gene – a gene is a sequence of DNA that codes for a molecule and/or that has a function.

Gene Drive mosquitoes (GDMs) – also called genetically engineered mosquitoes or transgenic mosquitoes – mosquitoes that have heritable traits derived using recombinant DNA technology, which alters the strain, line, or colony in a manner usually intended to result in a reduction of the transmission of mosquito-borne human diseases. GDMs are also likely to be characterised by the introduction of heritable marker traits to facilitate monitoring upon release into the environment.

Genotype – the genetic constitution of an organism.

Hazard – an event, activity or other cause of a negative consequence or impact identified in a risk analysis.

Infection incidence – the rate (number of cases per unit of population) at which new infections occur during a specific period.

Integrated vector management (IVM) – a rational decision-making process for the effective and efficient use of a combination of available resources in the management of vector populations to reduce or interrupt transmission of vector-borne diseases.

Pathogen – a microorganism that causes disease.

Phenotype – the observable characteristics of an organism based on genetic and environmental influences.

Regulation – an official rule to manage the conduct of those to whom it applies, usually developed from legal interpretations of legislation and implemented by government ministries or agencies.

Risk – an uncertain event or condition that, if it occurs, has a positive or negative impact on a project's objective such as time, cost, scope, quality.

Risk analysis – the process of prioritising and classifying risks and then determining which risks require the development of mitigating strategies and/or contingency plans. It reflects the project's tolerance for risk and defines thresholds and tolerance levels in areas such as cost, schedule, staffing, resources, quality etc., which, if triggered, may require the implementation of defined contingency plans.

Release – the process of placing GDM in the environment as part of the deployment of the technology.

Risk assessment – a methodological approach to define and characterise hazards and to estimate the exposure or likelihood of each hazard occurring as well as the potential adverse impact.

Risk management – the process of identifying and implementing measures that can be used to reduce risk to an acceptable level.

Risk communication – the process through which concerns and tolerance of risk are articulated by relevant stakeholders and results of its assessment and management are communicated to decision-makers and the public.

Traits – phenotypes that result from single or multiple genes and their interactions with the environment.

Vector mosquitoes – mosquitoes capable of transmitting a disease-causing pathogen.

[1] Reference to World Organization glossary

Executive Summary

Gene drive applications have been demonstrated in the laboratory to effectively suppress malaria vector populations and to alter the mosquitoes such that they are incapable of transmitting malaria parasites. Further tests are being planned to examine its field effectiveness in Africa. The technology could potentially constitute a high-impact, cost-effective and sustainable method for control and eradication of disease-transmitting mosquitoes. Since 94% of the world's malaria burden is currently in Africa, the continent is poised to be a major beneficiary of this technology, should it be proven effective. The spectrum of risks associated with the technology, however, remains less understood. As a result, any field testing or deployment will present unique regulatory, ethical and environmental concerns, which must be addressed to ensure its safe use.

The WA-IVM programme, an initiative of ECOWAS, has therefore developed a set of guidelines to support safe and responsible applications of gene drive mosquitoes. This specific guide focuses on mosquitoes containment procedures and contains recommendations for investigators, institutional biosafety committees, funding agencies, insectary managers and any other persons handling GDMs. The document gives an overview of how the GDMs must be handled in the laboratory and the safety equipment and facilities required for GD mosquitoes containment at different phases of testing both in the laboratory and the fields. The recommendations cover standard and specific practices inside laboratories, as well as management of outdoor containment facilities such as tents and outdoor cages. The principles of risk assessment and management are also covered to emphasise the importance of such assessments during containment.

All the practices highlighted in this guideline intend to fulfil the requirement of the GDM Biosafety levels (GDMBSL) 1 and 2.

Introduction

According to the WHO report of 2020, there were 229 million cases of malaria and 405,000 deaths. The WHO African region accounted for 94% of the disease burden. Nine of the ten most affected countries are in Africa, and Nigeria alone accounts for almost a quarter of all global malaria cases.

Current malaria prevention methods rely heavily on the use of insecticides. They include Indoor Residual Sprays (IRS) and Long-Lasting Insecticidal Nets (LLINs), and in a few cases, the use of larvicides. These tools have contributed to significant reductions in malaria cases and deaths since 2000, but recent evidence suggests that this progress may be levelling off or even slowing in some areas (5, 6). Some of the major challenges affecting malaria control efforts include inadequate financing, health system weaknesses such as those experienced in conflict areas, sub-optimal use of existing tools, the emergence of parasite resistance to antimalarial medicines and widespread mosquito resistance to insecticides.

In this context, significant improvements are necessary to accelerate malaria control and eradication efforts. This requires innovative approaches to complement current ones. ECOWAS has therefore created the WA-IVM Programme to address these needs. The main purpose of this initiative is to establish and operationalise a platform that will enable the region to build strong collaborations between multiple stakeholder groups (including health, biosafety, and other sectors) to effectively control malaria vectors. The WA-IVM programme will also equip the region to effectively apply existing vector control approaches as well as those that are on the horizon. The use of gene drives for controlling malaria has been adopted by the ECOWAS as a starting point.

Though research is still ongoing, Gene Drive technology has the potential to be a high-impact, cost-effective and durable method to control malaria transmission and could make a significant contribution to malaria eradication efforts. The current gene drive systems that have so far been tested inside laboratories have demonstrated the potential to spread beneficial traits through interbreeding populations of malaria mosquitoes. The approach works by either suppressing malaria vector populations or transforming the mosquitoes such that they can no longer transmit malaria parasites. However, some characteristics of this technology have raised concerns that necessitate careful consideration of the product development pathway. In particular, there have been concerns that such organisms might have unpredictable properties and potentially harmful effects on human, animal and environmental health. As a result of this, AUDA-NEPAD and WAHO are supporting the development of the appropriate guidelines for various phases of product development and testing, with the understanding that Africa stands to gain substantially if the technology is proven safe and effective.

These guidelines are applicable to all research and development activities of Gene Drive conducted inside governmental, non-governmental or private institutions. It is recommended that the guidelines be used in addition to relevant legislations and existing documents. This specific document deals with containment procedures and therefore outlines how the GDMs must be handled, the main safety equipment and facilities required for containment at different phases of testing, and the relevant risk assessment requirements for all the stages.

Background

In 2017, the AU adopted three reports of the Africa Union High Level Panel on Emerging Technologies (APET), one of which was on the Gene Drives for Malaria Control and Eradication in Africa. The AUDA-NEPAD, in collaboration with WHO and other partners, have since been planning the next steps to improve opportunities for harnessing these technologies safely to improve the health and wellbeing of Africans. Relevant guidelines are currently being developed to support this process.

This document presents the key guidelines and standards for the construction of containment facilities, equipment and operations when handling genetically modified mosquitoes and other mosquitoes. While the document focuses on preventing environmental harm from accidental release, the general containment principles for this class of mosquitoes are also applicable to other blood-sucking mosquitoes.

In 1964, the WHO defined genetic control as "the use of any treatment that can reduce the reproductive potential of the

insect by altering or replacing the hereditary material". This definition encompasses both categories of genetics-based strategies into which today's approaches fall, namely population suppression and population modification. The application of both strategies for mosquito control was imagined to be manipulations of existing phenotypes and genetic phenomena (7, 8). Early proponents of population suppression by genetic means envisioned releasing mosquitoes that would produce sterile offspring after mating with wild mosquitoes. Roles were proposed for genetic elements with interesting inheritance phenotypes such as sex-ratio distorters, which generate male bias in populations and could eventually lead to extinction.

The likelihood that a Gene Drive cassette would persist in the environment in the case of accidental release from laboratory containment, a test site or a rearing facility has been modelled for different drive mechanisms and found to depend variably on the number of mosquitoes released and the efficiency of the drive (9). The high efficiencies of CRISPR/Cas9 products require a reconsideration of the potential to control their activity during experimentation in the laboratory, where the accidental release may happen, but also in the wild once they are intentionally released for disease control. A high level of care when working with gene drive constructs is considered essential to the ethical development of the CRISPR/Cas9-based technology for all potential applications (10,11,12,13,14).

In addition to gene drive containment recommendations, several mechanisms to reverse a drive following the intentional releases are being developed. These strategies are particularly relevant for population modification strategies. While some GD population suppression strategies are also inherently self-limiting, gene-drive based suppression strategies are capable of wide geographic spread. The Cas9/sgRNA complex and HEGs have been proposed as the basis of both reversal mechanisms, including designs for synthetic constructs driven by another construct, even in several iterations (called "daisy chains"), in order to limit the spread of independent self-driving constructs in the case of an accidental release (15). Such systems have been demonstrated in yeast and modelled to provide a robust and containable drive mechanism (15).

Scope

The West Africa Integrated Vector Management (WA-IVM) Programme is an established programme of the Economic Commission of West African States (ECOWAS) to control and eradicate vector-borne diseases from the region. The purpose of this Programme is to establish and operationalise a platform that will enable the region to build a strong collaboration in the use of GDMs, focusing initially on the use of gene drive technology for controlling mosquito vectors. It will enhance the quality and consistency of procedures for the application of GDMs to ensure comparability of results and credibility of conclusions or decisions and guidelines relevant to these technologies. The purpose of this present document is to provide guidance for appropriate biosafety practices for handling GDMs in the laboratory or the field. Different levels of containment required for gene drive organisms are described. The guideline is purposefully broad and standard so that different users may adapt it to suit the different mosquito species or ecologies being targeted.

Containment Facilities

As described in the WHO Guidance Framework, testing of Gene Drive products begins with small-scale laboratory studies for efficacy and safety testing under appropriate containment conditions and operating procedures (WHO, 2021). This phase may proceed through testing in larger population cages within the laboratory setting, including large environmentally- controlled indoor spaces that aim to simulate a field setting[1].

GD mosquitoes Containment Levels in the laboratory

When GD mosquitoes are used, facilities, trained staff, and established practices must be in place to ensure adequate safety and the protection of the health and wellbeing of workers and the environment. This publication provides guidelines for laboratory work with GD mosquito vectors of pathogenic agents and has been prepared in response to concerns related to the consequences of an accidental release of mosquitoes. The guidelines are also in response to the question "what happens if the mosquitoes escape?" Suggested containment levels, therefore, address the question, "how do we prevent escape?" If working with a vector in a particular set of circumstances, a certain containment level may be recommended.

[1] https://www.liebertpub.com/doi/full/10.1089/vbz.2018.2431?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3Dpubmed

The Institutional Biosafety Committee (IBC) plays an essential role in establishing the appropriate GD mosquitoes containment level (GDMCL) in line with national legislation. It is responsible for reviewing research protocols and decides the appropriate level of containment at which the experiments must be performed. Where a mosquito is infected with an agent (Gene drive construct), the containment level required is automatically increased to at least that required for the agent. An example is the use of female mosquitoes to propagate malaria parasites. Furthermore, since any escape of even uninfected exotic mosquitoes must be prevented by all reasonable means, these are also handled at the GDMCL-2 level or higher. One advantage of working with certain mosquitoes is that the risk of release can be manipulated by, for example, performing relatively high-risk experiments during an unfavourable season when any escaped mosquitoes would quickly be killed by adverse environmental conditions. The IBC might use such biological considerations to "downgrade" a particular protocol from GDMCL-3 to GDMCL-2, providing those experiments are performed during a particular period. Documentation of the justification for this decision-making process should be prepared to ensure careful consideration of the risks.

It is important to develop a response procedure that is appropriate in case of an accidental release. The ideal response would be one in which all released GD mosquitoes are killed almost immediately after the escape. This may be impossible if the escaped GD mosquitoes get outside of the laboratory; hence the use of several barrier levels is recommended to maximise opportunities for locating and destroying the escapees.

GD Mosquitoes Containment Level 1 (GDMCL-1)

GD mosquitoes containment Level 1 (GDMCL-1) is suitable for work with uninfected mosquito vectors or those infected with a non-pathogen, including:

- mosquitoes that are already present in the local geographic region regardless of whether there is an active vector-borne disease transmission in the locality, and
- exotic mosquitoes that upon escape would be inviable or become only temporarily established in areas and not have active vector-borne disease transmission.
- The guidelines are organised in standard and specific practices of each containment level (13, 14, 16).

Standard Practices

Location of GD mosquitoes containment facility

Furniture and incubators containing GD mosquitoes are located in such a way that accidental contact and release are minimised. This may be achieved by locating mosquitoes out of the flow of general traffic, avoiding hallways, or placing them in closets.

Supply storage

The area is maintained to allow the detection of escaped mosquitoes. For example, materials unrelated to mosquito rearing and experimentation (e.g., plants, unused containers, clutter) that provide breeding sites and harbourages are minimised.

General mosquitoes eradication

Accidental sources of mosquitoes from within the insectary are eradicated. This may be accomplished by cleaning work surfaces after spillage of materials, including soil or water that might contain viable eggs. Pools of water are mopped up immediately.

Cleaning and disinfestation of primary containers

Practices should be in place such that mosquitoes do not escape by inadvertent disposal in primary containers. Cages and other culture containers are appropriately cleaned to prevent mosquitoes' survival and escape (e.g., heated to temperatures above the lethal temperatures or killed by freezing).

Construction of primary containers

Cages used to hold mosquitoes should prevent escape at all developmental stages. Screened mesh, if used, must be durable and of appropriate mesh size to prevent escape. Non-breakable cages are recommended. Bags and rearing trays effectively prevent leakage and escape.

Disposal of mosquitoes

All wastes from the insectary (including carcasses and rearing medium) should be safely 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. Autoclaving or incineration of material infected with a non-pathogen is recommended. The material must be killed with hot water or frozen before flushing down drains.

Identification of mosquitoes and labelling of primary containers

Mosquitoes must be adequately identified and labelled as follows: name of species, strain/origin, date of collection, responsible investigator, etc. The label must be firmly attached to the container (and cover if removable). Vessels containing stages with limited mobility (e.g., eggs, pupae, hibernating adults) must be securely stored.

Prevention of accidental dispersal

The institution must ensure appropriate precautions to prevent transport or dissemination of mosquitoes from the insectary on the clothing or via the sewer.

Pest exclusion programme

A programme must be set up to prevent the entry of wild insects (e.g., houseflies, cockroaches, spiders) and rodents. This effectively precludes predation, contamination and possible inadvertent infection.

Monitoring of escaped mosquitoes

Investigators must assess whether escapes are occurring. An effective mosquito trapping programme in and around the facilities is recommended to monitor the escape prevention programme.

Source and harbourage reduction

Harbourage and breeding areas must be reduced as appropriate. Furniture and racks are minimised and can be easily moved to permit cleaning and location of escaped mosquitoes.

Microbiological and medical sharps

Syringes that re-sheath the needle, needleless systems, and other safety devices are used when appropriate. Plastic-ware is substituted for glassware whenever possible.

Notification and signage

Persons entering the area are made aware of the presence of mosquito vectors through appropriate signs and notification. In addition, records of people visiting the facility should be captured in a logbook provided.

Special practices for handling vertebrate animals

IBC approval

IBC approval is required for non-exempt recombinant DNA protocols. Investigators should consult with their institutional research oversight office if vertebrate animals will be used to feed hematophagous mosquitoes. The requirement for IBC review is an institutional decision, although highly recommended.

Housing of vertebrate animals

Animals not required for culture should not be made accessible to the mosquitoes. Animals used as hosts or blood sources may be housed within the insectary but must be adequately protected from access by escaped mosquitoes.

Containment during blood-feeding

Mosquitoes fed on host animals are prevented from accidental transfer to host cages. When handling/removing animals after exposure to mosquitoes, precautions must be taken to prevent mosquitoes from escaping through screens, covers, and by-flying. Host animals are inspected closely to remove any attached mosquitoes (e.g., concealed in fur, ears, crevices), and the primary container is sufficiently robust to prevent escape during feeding.

Blood source

The blood source is considered as a source of inadvertent mosquito 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. The use of blood from animals or humans whose disease status is uncertain must be avoided.

Handling of escaped mosquitoes

Escaped mosquitoes are killed or collected and properly disposed of.

Reporting accidental release

The Insectary Director is notified promptly of the accidental release of vectors.

Safety Equipment (Primary Barriers)

Gloves

Gloves must be worn when handling host animals or blood used to feed the mosquitoes.

Torso apparel

White laboratory coats, gowns, and/or uniforms must always be worn in the insectary when handling blood and vertebrate animals.

Mosquito-specific personal protective equipment

Personal protective equipment must be worn as appropriate, e.g., respirators for mosquito-associated allergies, particle masks, head covers etc.

Facilities (secondary barriers)

Location of insectary

The insectarium must be located out of the flow of general traffic, avoiding hallways or placing mosquitoes in closets. The area must be designed to allow the detection of escaped mosquitoes. For example, the walls of the facility must be painted white or with a contrasting colour to the mosquitoes that are kept in the facility.

Insectary doors

Door openings must be covered by rigid panels, glass, screens, plastic sheets or cloth to minimise escape and entry of mosquitoes.

Insectary windows

Windows must be covered with mesh or screen to effectively prevent the escape of mosquitoes. All air-conditioning inlets and outlets must be covered with suitable-sized mesh to prevent mosquitoes from passing through. The GDMCL-1 facility must be fitted with a suitable electric insect-control unit or an appropriate mosquito trap.

GD mosquitoes Containment Level 2

GD mosquitoes Containment Level 2 (GDMCL-2) must be practised if working with exotic and indigenous mosquitoes infected with GDMBSL-2 agents associated with animal and/or human disease or that are suspected of being infected with such agents. GDMCL-2 builds upon the practices, procedures, containment equipment, and facility requirements of GDMCL-1. It is more stringent in physical containment, disposal, and facilities design. Moreover, access is more restricted than GDMCL-1. The decision to cultivate infected exotic mosquitoes under GDMCL-2 conditions in active transmission areas or in cases in which establishment is a possibility requires that measures that otherwise would only be recommended or preferred must be met (13, 14, 15, 16).

Standard Practices

Location of mosquitoes

Furniture and incubators containing mosquitoes are located in such a way that accidental contact and release by laboratory personnel, custodians, and service persons are unlikely. This may be achieved by locating mosquitoes in dedicated rooms, closets and incubators located out of the traffic flow or similar measures.

Supply storage

The area is designed and maintained to enhance the detection of escaped mosquitoes. Equipment and supplies not required for the operation of the insectary must not be in the insectary. All supplies for insect maintenance that must be kept within the insectary should be in a designated area and not on open shelves. It is recommended that closed storage room cabinets with tight-fitting doors or drawers be used. Doors and drawers must be opened only for access. Insect diet must be kept in sealed containers. Blood for feeding must be kept under refrigeration.

Cleaning and disinfestation of primary containers

In addition to cleaning cages and culture containers to prevent mosquito escape as in GDMCL-1, containers are disinfected chemically and/or autoclaved if used for uninfected material. Autoclaving or incineration of primary containers is recommended for containers holding infected material.

Construction of primary containers

Cages used to hold mosquitoes are non-breakable and screened with an appropriate mesh size to prevent escape. Containers are preferably autoclavable or disposable. Openings designed to prevent escape during the removal and introduction of mosquitoes are recommended.

Disposal of mosquitoes

In addition to standard GDMCL-1 disposal practices, autoclaving or incineration of mosquito materials is recommended. Infected mosquitoes are autoclaved or incinerated.

Isolation of uninfected mosquitoes

The spread of agents to uninfected mosquitoes is usually low risk, given that most infections occur via blood-feeding. Containers must be clearly marked to easily distinguish infected mosquitoes from uninfected ones. It is good practice to keep infected mosquitoes in a separate room, if possible, to prevent them from being mistaken as uninfected.

Identification and labelling of primary containers.

Refer to GDMCL-1

Prevention of accidental dispersal

Personnel who handle cultures and infected mosquitoes must wash their hands before leaving the insectary. Care must be taken to avoid the dispersal of viable life stages into the drainage system. No infected material must be disposed of through the sewer. In this case, all material must be decontaminated by heating or freezing, preferably by autoclaving or incineration. Air curtains are recommended as appropriate. Personal protective equipment that is reused (laboratory coats, gowns) should be checked for infestation before exiting the insectary.

Pest exclusion programme

Refer to GDMCL-1

Monitoring of escaped GD mosquitoes

Investigators must assess whether escapes are occurring by instituting an effective mosquito trapping programme at the facilities to monitor the escape prevention programme. Oviposition and light traps for mosquitoes are recommended. In cases where exotic mosquitoes are used, exterior monitoring is recommended. Records of exterior captures must be carefully maintained.

Source and harbourage reduction

Harbourage and breeding areas must be eradicated. Furniture and racks should be minimised and be easily moved to permit cleaning and location of escaped mosquitoes. Equipment in which water is stored or might accumulate (e.g., humidifiers) must be screened to prevent mosquitoes' access or contains chemicals to prevent mosquitoes' survival.

Microbiological and medical sharps

Refer to GDMCL-1

GD mosquitoes sharps

In addition to minimising GD mosquitoes' sharps, these items should be restricted for use in the insectary if infected materials are used.

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 soil or water that might contain infectious agents or eggs).

Notification and signage

Persons entering the area must be made aware of the presence of mosquito vectors. If infected material is present, a GDMBSL-2 biohazard sign is posted on the entrance to the insectary listing all species handled within and is updated whenever new species are introduced, or pathogenic infectious agents are present. The hazard warning sign identifies the mosquito species, agent(s) known or suspected to be present, names and telephone numbers of the responsible person(s) and any special requirements for entering the insectary (e.g., the need for immunisations or respirators).

Procedure design

All procedures are carefully designed and performed to prevent escape of mosquitoes.

Safety manual

A safety manual must be prepared, approved by the IBC, and adopted. The manual must 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 must be advised of special hazards and be 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 must receive annual updates and additional training as necessary for procedural or policy changes. Records of all training must be carefully maintained.

Medical surveillance

An appropriate medical surveillance programme must be put in place. All personnel must receive appropriate immunisations or tests for the agents handled or likely to be present. When appropriate, a serum surveillance system is implemented. Personnel must be aware of the symptoms of infection and the procedure to follow in reporting these. In general, persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous (e.g., the immunocompromised) are not allowed in the insectary unless special personal protection procedures are in place to eradicate extra risk.

Access restrictions

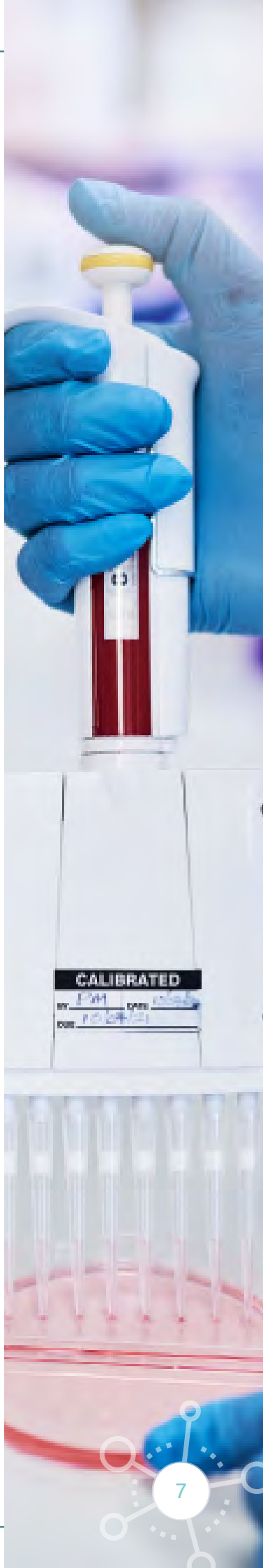
Routine access is limited to trained persons and accompanied guests. Service persons must be made aware of the hazards present and the consequences of mosquitoes release and contact with agents that may be present.

Special mosquitoes handling containers and areas

Infected mosquitoes are prevented from being released into the laboratory area. This may be accomplished by secure glove boxes, biosafety cabinets, custom handling trays etc. These may vary from GDMBSL recommendations as far as necessary to safely contain both the mosquitoes and any agent. Such modifications must be made only in consultation with experts in handling the specific types of infected mosquitoes and biosafety experts. A dedicated area for handling infected material is recommended. This is preferably a separate cubicle, walk-in incubator, or screened room.

Safe transport in the laboratory

All infectious and potentially infectious samples are collected, labelled, transported, and processed in a manner that contains and prevents transmission of the agent(s). Transfer of mosquitoes between manipulation and holding areas is in non-breakable secure containers.



Special practices

The section aims to stress some special practices for the laboratory planning to use vertebrate animals for blood-feeding the mosquitoes under containment. A list of minimum requirements is provided.

IBC Approval

As for GDMCL-1, microbial agents classified at BSL-2 require at the minimum registration with the appropriate institutional entity. Many institutions require IBC review and approval before starting any work. In the institutions where these administrative entities are absent or less formally structured, this guidance may not easily be applied. In such cases, PIs and department leaders could institute site-specific policies of their own to ensure the safety of researchers and the surrounding community.

Housing of vertebrate animals

Other animals are not accessible to the mosquitoes. Animals used as hosts or blood sources generally are not housed with mosquitoes. If present, they are adequately protected from access by escaped mosquitoes, and protocols are approved by the IBC.

Containment during blood-feeding

Recommendations for GDMCL-1 containment of mosquitoes during blood-feeding must be stringently assured by special practices and container design.

Blood Source

Refer to GDMCL-1

Handling of escaped mosquitoes

Loose mosquitoes must be killed and disposed of or recaptured and returned to the container from which they escaped. Infected mosquitoes must not be killed with bare hands and must be transferred using filtered mechanical or vacuum aspirators.

Reporting Accidental Release

A release procedure is developed and posted. This includes contacts and immediate mitigating actions. Accidents that result in the release of infected mosquitoes from primary containment vessels or that result in overt exposure to infectious material must be reported immediately to the Insectary Director, who is responsible for ensuring that appropriate and documented action is taken to mitigate the release. Location, number, and type of material are prominently posted until the source is eradicated. Follow-up medical evaluation, surveillance, and treatment are provided as appropriate, and written records must be maintained.

Movement of equipment

All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary and before removal from the insectary.

Safety Equipment (Primary Barriers)

Eye and Face Protection

Appropriate face/eye and respiratory protection must be worn by all personnel entering the insectary.

Gloves

Gloves must be worn when handling potentially infected mosquitoes, blood, and associated equipment and when contact with potentially infectious material is unavoidable.

Torso apparel

White laboratory coats, gowns, and/or uniforms must always be worn in the insectary when handling blood, vertebrate animals, and infected materials.

Personal clothing

Clothing must be minimised in exposed skin (e.g., skirts, shorts, open-toed shoes, sandals, tee shirts are inadvisable) since this can increase the risk of attracting and being bitten by loose mosquitoes.

Mosquito-specific personal protective equipment

In addition to GDMCL-1 measures, personal protective equipment is used for all activities involving manipulations of infected or potentially infected mosquitoes.

Facilities (Secondary Barriers)

Location of insectary

The insectary is separated from areas that are open to unrestricted personnel traffic within the building. It is recommended that this be accomplished by at least two self-closing doors that prevent the passage of the mosquitoes. Increased levels of physical isolation are recommended, e.g., separate buildings, wings, suites.

Insectary doors

Recommended entrance to the insectary is via a double-door vestibule that prevents flying and crawling mosquitoes escape. For example, the two contiguous doors must not be opened simultaneously. Internal doors may open outwards or be sliding but should be self-closing and must be kept closed when mosquitoes are present. Additional barriers (e.g., screened partitions, hanging curtains) are highly recommended.

Insectary windows

Windows are not recommended, but if present, they should be those that cannot be opened and must be well sealed. Windows must be resistant to breakage (e.g., double-paned or wire-reinforced).

Vacuum systems

If a central vacuum system is installed, each service outlet is fitted with suitable barriers/filters to prevent escape of mosquitoes. Filters are installed to permit decontamination and servicing. Other vacuum devices are appropriately filtered to prevent the transfer and exhausting of mosquitoes.

Interior surfaces

The insectary is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior walls are light-coloured

so that loose mosquitoes can be easily located, recaptured, or killed. Gloss finishes, ideally resistant to chemical disinfectants and fumigants, are recommended. Floors are light coloured, smooth and uncovered. Ceilings are as low as possible to simplify the detection and capture of flying insects.

Floor drains

Floor drains should be modified to prevent the accidental release of mosquitoes and agents. If present, traps must be filled with an appropriate chemical treatment to prevent the survival of all mosquito stages (e.g. mosquito larvae).

Plumbing and electrical fixtures

Internal facility appurtenances (e.g., light fixtures, pipes, ducting) are minimal since these provide hiding places for loose mosquitoes. Penetrations of walls, floors, and ceilings are minimal and sealed/caulked. Ideally, light fixtures are flushed with the ceiling, sealed, and accessed from above.

Heating, ventilation and air conditioning

Ventilation is appropriate for mosquito maintenance but must not compromise containment of the agent or mosquitoes. For example, exhaust air should be discharged to the outside without being recirculated to other rooms; appropriate filter/barriers should be installed to prevent the escape of mosquitoes; the direction of airflow in the insectary should be inward; a progressively negative pressure gradient should be maintained as the distance from the main entrance increases; fans located in the vestibule and internal corridor can be used to help prevent the escape of flying mosquitoes; air curtains should be located in vestibules and doorways.

Sterilisation equipment

An autoclave or other sterilising equipment must be conveniently located in rooms containing mosquitoes within the insectary building.

Sink and shower

The facility must have a hand-washing sink with hot water and with suitable plumbing to prevent escape of mosquitoes.

Illumination

Illumination is appropriate for mosquito maintenance but must not compromise mosquito containment, impede vision, or adversely influence the safety of procedures within the insectary. Lighted (or dark) openings that attract escaped mosquitoes must be avoided.

Facility compliance monitoring

The facility must be evaluated annually for compliance with the GDMCL-2 level. The Principal Investigator or Insectary Director must inspect the facility annually to ensure that alterations and maintenance do not compromise the containment characteristics.

Large cage trials of GD mosquitoes

Field containment refers to those practices that prevent the unplanned or uncontrolled release of GDM into the field. This must be accomplished by good work practices, strict adherence to standard operating procedures (SOPs), trained personnel and physical confinement within an enclosed structure. The physical containment afforded by cages may be augmented by biological containment (the use of organisms that have reduced ability to survive or reproduce in the environment) and/or ecological containment (geographic, climatic, or spatial isolation), which limit the spread of organisms into the environment.

The establishment of a site for contained field trials will likely require ready access to a field laboratory facility for the husbandry of mosquito colonies, monitoring analyses, and other relevant research. This field laboratory must receive biosafety inspection and approval from the relevant academic or public health institution. SOPs will be required for the safe transport of mosquitoes between the field laboratory and field cage.

Location of field containment

This must be based on agreement among the investigators, local communities, and the appropriate regulatory, public health, and government bodies. The criteria of selection of this location may include ecological relevance; existing laboratories or other infrastructure that can be modified into a field laboratory; relative isolation from adjacent

communities (but not so remote that it will complicate simultaneous field research with local mosquitoes populations in the case of urban vectors); relative ease of securing the area; and consent of the local communities, local authorities and appropriate regulatory/ government agencies.

Cages must be built on stable ground. Enclosures must be positioned so as to prevent the risk of damage to the structure from falling tree limbs, flooding, fire, and other such disasters. A mosquito habitat-free zone around the structure would further minimise the survival and establishment of escaped mosquitoes.

Materials and Design

Cages must have mesh on the sides and ceiling. Commercially available greenhouses or screen houses may be used with modifications to ensure improved security, adaptation to environmental conditions, or protection against natural hazards such as storms or earthquakes. For example, for most mosquitoes, double-wall siding with 120–200 mesh/ square inch (1.2 mm x 1.2 mm) is adapted. The space between the layers of the mesh can be wide enough (0.5 –1.0 m) to allow monitoring personnel to walk between layers to check the cage integrity and set traps to monitor for escaped mosquitoes.

The mesh selected must be resistant to ripping, rusting, tearing, and stretching with good KLY ratings (a measure of ultraviolet radiation energy per cm2 area per year). This parameter will provide an estimate of the life span of the mesh. Rainfall must also be taken into consideration in the choice of ceiling materials.

The design of the cage foundation is another critical aspect of the construction. Better simulation of the natural environment will be achieved if the floor of the cage is composed of soil with locally appropriate vegetation. Soil drainage of rainwater is an important consideration, and an open bottom would facilitate more natural drainage of the ground within cages.

Drainage and the security of cage drains are important design features. Although adequate drainage is required to maintain the habitat, drains provide an opportunity for the entrance of animals. Careful thought should be given to methods for blocking ingress and egress through drains (such as placement of water-permeable barriers and plumbing traps within the drainage pipes).

Security of the facilities

It is recommended that the cages be protected from trespassing by pest animals using a security fence. In situations where additional precautions are needed, the fence may be lined with razor wire along the top, and page wire can be used to exclude animals. Trapping and regular monitoring for rodents and other vermin must be conducted. Partial walls or trenches around the cage can provide enhanced protection against the incursion of animals. Signs must be placed appropriately around the perimeter. A detailed security response plan must be in place describing responsibilities and actions to be taken in the event of a breach.

Operating procedures and training

SOPs should provide training on scientific needs, maintenance, and safety procedures for staff involved in all aspects of the trial. The training should be repeated regularly and should include a plan for routine oversight of employee performance. The rationale for precluding the entry of other organisms into the cage and preventing the escape of mosquitoes must be thoroughly explained to all employees, and they must then be questioned to ensure understanding.

Monitoring and surveillance

Ongoing surveillance for the inadvertent escape of mosquitoes from inside the enclosures into the surrounding environment is essential. All feasible efforts must be made to ensure containment, accompanied by active monitoring to facilitate detection should GD mosquitoes escape into the immediate and adjacent areas where the cage studies are being conducted. Monitoring methods must be tested using native mosquitoes from the surrounding area during the pre-trial testing phase, prior to experiments involving GD mosquitoes. Appropriate indicators and analysis plans should be put in place to interpret the data obtained from the monitoring and surveillance programme.

Monitoring of containment must be done by visually examining cages regularly for structural damage and mesh integrity following a detailed checklist for each cage area. The record must be archived.

Intervention and remediation strategies

SOPs must be in place in advance of the study, describing in detail the actions to be undertaken in the event of a pending or proven breach of containment. The SOPs must be available in the language(s) of the local employees, and regular testing or simulations should be conducted to ensure that all employees are familiar with the criteria for decision-making and the procedures that must be implemented. It is essential that all employees know who is responsible for decision-making in this situation and that they are able to contact the responsible individuals without delay. Local public health and vector control authorities, as well as community leaders, must be consulted in the development of these SOPs, and the plans must be shared with those living in the surrounding area as part of the community engagement process.

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