Safety measures for work on genetically modified mosquitoes in containment

Laboratory containment and safety

Target Malaria laboratories and insectaries are built to comply with recognised international standards for arthropod (including mosquito) containment. Our genetically modified mosquitoes (gene drive and non-gene drive) are housed in containment facilities that must also meet national guidelines for biosafety for contained use permits to be granted. These guidelines ensure that there are multiple measures to prevent the unintended release of genetically modified mosquitoes into the environment. The research protocols we follow and type of mosquitoes we study determine the precise safety level at which our laboratories operate. Specifically, there are two types of safety guidelines:

Arthropod Containment Level (ACL)	Levels of precaution designed specifically for laboratories working on arthropods, such as mosquitoes, to prevent their escape from containment facilities. ACL-1 is the lowest risk category while ACL-4 is the highest.
Biological Safety Level (BSL)	Levels of precaution required in any laboratory to avoid biological risk to workers, with BSL-1 being the lowest risk category and BSL-4 being the highest risk category.

Laboratories and insectaries that work only with native local wild mosquitoes require ACL-1 containment. This is because the mosquitoes are already present in the local geographic region and escape would not result in the establishment of a new arthropod in that area. The native local mosquitoes are brought into the insectary as eggs and therefore do not carry any pathogens. The mosquitoes do not pose a risk to human health.

Under ACL guidance, all types of genetically modified mosquitoes require ACL-2 containment, and this applies to gene drive arthropods too. This is the level at which all Target Malaria's mosquito laboratories and insectaries operate. The modified mosquitoes that we work with do not normally contain any pathogens and therefore do not pose a risk to human health so that, in most cases our laboratories and insectaries are equivalent to BSL-1. The only exception to this is where we need to test the ability of the modified mosquito to transmit diseases like malaria; such experiments are conducted under more stringent safety measures in BSL-2 facilities.

Safety measures to prevent escapes

Our laboratories and insectaries are built to comply with recognised international standards for arthropod containment. Our laboratories and insectaries in Africa are single use facilities where no other projects operate and access is restricted to only those project team members that need entry. Our teams follow well-established methods[1] for safely handling and working with mosquitoes to avoid escapes. They are trained and competent in good insectary management, follow Standard Operating Procedures (SOPs), and monitor laboratory and insectary conditions regularly.

There are several built-in mechanisms to catch mosquitoes that may escape from their cage during handling. For example, as the insectaries are housed inside laboratory buildings, if a mosquito flies outside its cage during handling, it remains within the contained facilities. The facilities also have a range of other precautionary measures, such as mosquito traps at key points in the insectary, secured double doors, fans blowing air away from doors, water filters to catch eggs, and other built-in mechanisms to contain mosquitoes within the facility.

In the unlikely event that mosquitoes were to escape from the insectary building, our teams have developed, and are trained to implement, a rapid response plan to address the breach in containment. In Africa, these include monitoring populations of wild mosquitoes outside the laboratories and local weather conditions for breeding and may include spraying resting sites in the immediate area around the insectary with insecticide. Target Malaria is working with local authorities and with residents around the facilities to ensure their agreement and familiarity with routine insectary monitoring and with these response plans. In Europe, the mosquitoes would not be able to survive outside of their natural environments, so the local conditions create an ecological barrier.

Transport of mosquitoes between countries: safety measures

Target Malaria transports genetically modified mosquitoes from Europe and the United States to Africa. Importation of any genetically modified mosquitoes only takes place with the permission of the national regulatory agency. In Africa, such an import can only be approved with the support of the local communities.

Mosquitoes are transported between laboratories as eggs, which need high humidity to survive. The eggs are contained in triple sealed containers that are specifically approved for shipping. In the unlikely event that the packages were broken during shipping, the eggs would dry out and die. Target Malaria only uses commercial carriers with IATA certification.

Preparedness of our insectaries in Africa

Target Malaria has a detailed process to prepare its teams for working with genetically modified mosquitoes. This process is referred to as "Facilities readiness" the details of which have been published by the project[2]. As part of this facilities readiness our teams work firstly with a non-genetically modified mosquito that has a naturally occurring colour variation making it easier to identify. This step builds the teams' confidence and competence with a non-standard mosquito.

The laboratories and insectaries comply with existing relevant national regulations or certification requirements and are also audited internally against international guidance and consensus on what is appropriate to this containment level to ensure good laboratory standards are met and maintained.

Throughout the whole process the project teams engage with communities around the facilities so that they understand the work that Target Malaria is doing.

Considerations for Gene Drive Mosquitoes

Target Malaria is currently developing and studying gene drive mosquitoes at its insectaries in the UK (Imperial College London), Italy (Polo d'Innovazione di Genomica, Genetica e Biologia) and in the United States (CDC Foundation). The current legislation in Europe does not distinguish containment measures for gene drive organisms over genetically modified non-gene drive ones[3]. Therefore, all facilities working with gene drive mosquitoes require ACL2 containment.

While none of the Target Malaria insectaries in Africa are currently working with gene drive mosquitoes, nor will be for several years, the facilities already conform to ACL2 requirements because they are working with genetically modified mosquitoes.

Before any genetically modified mosquitoes with a gene drive element are shipped to our insectaries in Africa a dossier to request a permit for contained use would be submitted to the national competent regulatory body for consideration. A contained use permit is the first step in working with a genetically modified mosquito, whether it has a gene drive or not. In addition, the local communities would be consulted and invited to give their consent for this phase of the work to take place.

1 Mumford *et al.*, Maintaining quality of candidate strains of transgenic mosquitoes for studies in containment facilities in disease endemic countries, *Vector-Borne Zoonotic Diseases*, 2018.

2 Quinlan *et al*, Containment Studies of Transgenic Mosquitoes in Disease Endemic Countries: The Broad Concept of Facilities Readiness, *Vector-Borne Zoonotic Diseases*, 2018.

3 European Food Safety Agency (EFSA), Scientific Opinion, Adequacy and sufficiency evaluation of existing EFSA guidelines for the molecular characterisation, environmental risk assessment and post market environmental monitoring of genetically modified insects containing engineered gene drives, *EFSA Journal 2020;18(11):6297, doi: 10.2903/j.efsa.2020.6297*