

# *Anopheles* Mosquito Collection, Storage and Transport in Ethanol

Guidance

**MalariaGEN**  
VECTOR OBSERVATORY



# MalariaGEN

## VECTOR OBSERVATORY

**Version 4**  
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### **About this document**

This guidance was prepared by the MalariaGEN Resource Centre members based at the Wellcome Trust Sanger Institute, UK, to assist our partners collecting and transporting whole mosquito carcasses, for the purposes of whole-genome sequencing of malaria vectors and any malaria parasites infecting the mosquitoes.

### **For more information, contact:**

[support@malariagen.net](mailto:support@malariagen.net)

Malaria Genomic Epidemiology Network (MalariaGEN)

<https://www.malariagen.net>

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## 1. SCOPE

This guidance was prepared by the MalariaGEN Resource Centre members based at the Wellcome Sanger Institute, UK, to assist our partners collecting mosquito carcasses from the field, for the purposes of sequencing *Anopheles* vectors and *Plasmodium* parasites.

This document was prepared from laboratory protocol; T112\_LAB\_SOP0001 v3.5 October 2020

## 2. MATERIALS REQUIRED:

2.1 Reagents and Materials		
<b>Reagents</b>		
80% (v/v) Ethanol solution (or as high a concentration as available)		
<b>Materials</b>	<b>Supplier</b>	<b>Cat. No.</b>
ABgene SuperPlate 96 well PCR plate	ThermoFisher Scientific	AB2800
PCR tube 8-well domed cap strip	Fisherbrand	11859251
Tweezers		
Parafilm® M Laboratory sealing film		
Cling film or Saran wrap		
Bubble wrap sheet or sleeves		
Card typically from laboratory supplies boxes		
Small beaker		
Tissue		
Pencil and/or biro		
Masking tape, autoclave tape, electrical tape or equivalent – NOT Sellotape		

2.2 Equipment
200 µl pipette
Filtered tips of various volumes

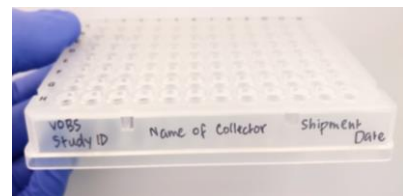
## 3. METHODOLOGY

### 3.1 How to collect *Anopheles* carcasses in 96-well plates in ethanol

3.1.01 Using a pencil, label one long side and one short side of the 96-well collection plate with (**see note 1**):

- VOBS study ID
- Name of project lead
- Plate number
- Shipment date

If barcodes are provided, attach to front of plate (i.e. row H side) and ensure the short side is also pencil-labelled



\* if you don't have a study ID please contact [support@malariagen.net](mailto:support@malariagen.net)

3.1.02



Fill each of the required wells with 150µl of 80% ethanol (**see note 2**).

**Leave wells G12 and H12 EMPTY.**

3.1.03 Due to using the 8-well domed cap strips it is recommended that the mosquitoes are added by column.

Using clean/sterile tweezers, place ONE whole mosquito (head, thorax and abdomen) into each well of the 96-well plate, making sure each mosquito body is completely submerged in the liquid.

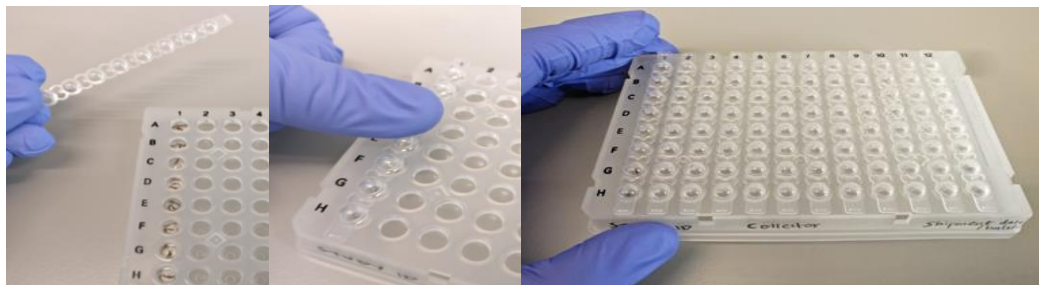
Clean your tweezers between each mosquito (**see note 3**).



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3.1.04 Once a column has been completed;

Place the 8-well domed cap strip onto a column of the plate. To seal, press the caps down onto each well until you hear a click, and they are tightly sealed.



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3.1.05 Repeat step 3.1.04 for every filled (or partially filled) column of wells. Following this, the plate is now ready to be packaged for shipping. **See Appendix 1 for troubleshooting tips.**

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## 3.2 Packaging the 96-well plates

3.2.01 Cut out a thick piece of card to the approximate size of the plate (12cm x 8cm), then place it on top of the sealed plate.

Make sure that the cardboard does not overhang the plate. This is vital for applying even pressure to the 8-well domed cap strips on the plate.



3.2.02 Securely tape the piece of card to the plate. At this point, double check that you have labelled the plate correctly as instructed in 3.1.01.






3.2.03 Take a large piece of parafilm and wrap it tightly around the plate several times to create a secure bundle.



3.2.04 Wrap a large piece of cling film (standard food grade) around the padded parafilmed plate several times until it is tight and secure.





<p>3.2.05</p>	<p>Wrap the plate in a piece of kitchen roll or other absorbent material to mop-up any leaks if they occur. Place the plate in a bubble-wrap sleeve, or just wrap in bubble-wrap, and tightly seal its open edges with adhesive tape</p>	
<p>3.2.06</p>	<p>Label this, using a biro or pencil, with:</p> <ul style="list-style-type: none"> <li>• VOBS Study ID</li> <li>• Date of Shipment</li> <li>• Plate ID</li> </ul> <p>NB: Labelling needs to be resistant to ethanol</p>	
<p>3.2.07</p>	<p>Place all of your wrapped mosquito plates into a large re-sealable/Ziploc bag. This sealed bag can now be handed over to your local shipping agency for boxing-up and shipping.</p> <p>You will also be required to provide information on the contents of the package such as the ethanol and volume.</p> <p><b>For some guidance, please see Appendix 2 for IATA transport regulations.</b></p>	

3.2.08	<p>Shipping address:</p> <p><b>MalariaGEN Team 112, c/o Eleanor Drury Sulston Building Wellcome Sanger Institute Hinxton, CB10 1SA, United Kingdom</b></p>	<p>Email: <a href="mailto:samples@malariagen.net">samples@malariagen.net</a></p> <p>Phone: + 44 - (0)1223 - 494994</p>
3.2.09	<p>Please email any postal/courier tracking information as soon as possible, thank you.</p>	
3.2.09	<p>Fill out the sample information sheet (SIS) provided, with details including sample ID, well position, plate ID, study ID, collection date and location.</p> <p>If you have not yet received a SIS or for any other queries, please contact:</p> <p><a href="mailto:samples@malariagen.net">samples@malariagen.net</a></p> <p>Furthermore, the SIS does provide more detailed instructions and guidance on how to complete it as well as which fields are required and optional</p> <p>Malaria Genomic Epidemiology Network (MalariaGEN)</p> <p><a href="https://www.malariagen.net">https://www.malariagen.net</a></p>	

## Appendix 1: Notes and Troubleshooting tips

If these notes do not answer your query please email us at;  
[support@malariagen.net](mailto:support@malariagen.net)

### 1. Labelling plates.

We recommend labelling the plates directly with a pencil because this is less likely to be affected or smudged by the alcohol used for filling the plates (however the ethanol may get onto the plate sides). If you are having trouble with this then you can request some plate barcodes from us to use instead (please email [samples@malariagen.net](mailto:samples@malariagen.net) with your study\_id, how many barcodes you require, and a mailing address).

Alternatively,

- we suggest cutting strips of masking tape/autoclave tape or something similar that you can stick onto the side of the plate. Then use a biro.
- after you have completed the plate and before you wrap it, you can put a piece of tape around the plate and then label it with the plate id using a biro.

The key here is to label the plate in a way that will not be affected by ethanol.

### 2. Pre-filling plates with ethanol.

Filling all wells on a plate with ethanol may result in some evaporation. We do not believe that this is a problem providing the specimens are fully submerged when you add them to the wells. If you are concerned about this then we recommend that you work column by column; add ethanol to all wells of a single column, then add the specimens and put the cap-strip on. Then move on to the next column.

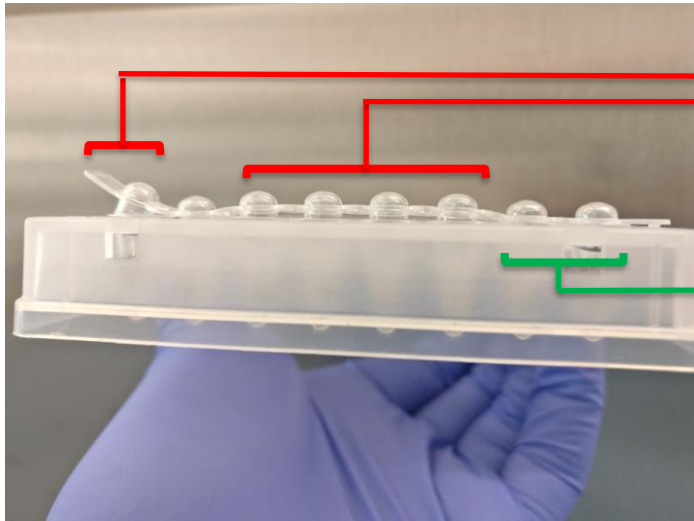
### **3. Tweezers must be cleaned between handling each mosquito sample.**

We suggest using two washes with either ethanol or water. Prepare a small beaker of your cleaning solution and dip the tweezers into this. Swirl around for a few seconds. Dab the tweezers on to a sheet of absorbent material (tissues for example) to remove excess moisture. It is not necessary for the tweezers to be completely dry before moving onto the next specimen. Otherwise you can wave the tweezers in the air, or wipe them with clean tissue.

If you have several sets of tweezers you could leave them standing in your 'cleaning' solution while you select another set for the next specimen.

#### 4. Strip caps

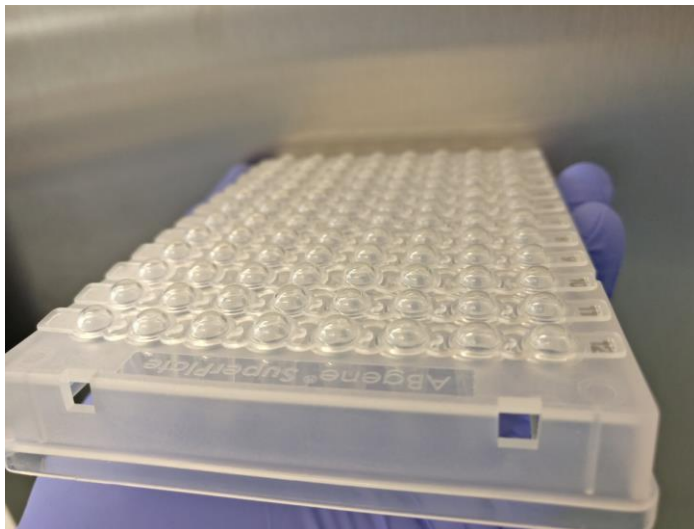
**Make sure the strip-cap seals are well secured and pressed flat into each well.**



Well caps not tightly pressed in and prone to sample leakage



Wells securely sealed



## Appendix 2: Shipping Information – Ethanol

Please note that the original documentation for IATA or equivalents should always be consulted for the avoidance of doubt. Courier companies should be able to answer such queries for the latest information and detail. IATA regulations are updated yearly.

The following has been extracted to provide guidance.

<https://www.iata.org/en/publications/dgr/>

### Guidance on IATA transport regulations 2019:

According to IATA, ethanol is classified as a ‘Class 3 Flammable liquid (e.g. lighter fluid, petrol)’ under the UN classification scheme. The majority of authorities use the same packaging regulations and classifications. However, they may have slightly different wording, but essentially require ethanol to be treated in the same manner. However, it is important to check with your local authorities and courier company for confirmation on regulations, shipment and labelling.

Under the UN1170 category for Ethanol (Ethyl Alcohol), the “Max volume” of ethanol that can be shipped per outer package (i.e. shipping box) is 1 Litre. However, for each individual inner package (i.e. a 96 well plate), the maximum volume is 30ml. In the instructions above where we suggest using 150uL per well, the total volume of ethanol/liquid is no more than about 14mL per plate, notwithstanding the actual concentration of ethanol used.

Assuming that 150uL per well has been used and all plate wells are used, this means that up to about 60 plates can be shipped in one ‘outer’ package.

150ul x 94 wells (G12 and H12 left empty) = 14.10 mL per plate.

1000 mL / 14.10 mL = 71 plates

The following UN classification of ethanol can be found at the website below:


<https://adrbook.com/en/2017/UN/1170>

Below are the relevant sections:

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UN No.	Name and description	Class	Classification code	Packing group	Labels	Special provisions	Limited and excepted quantities		Packaging			Portable tanks and bulk containers	
									Packing instructions	Special packing provisions	Mixed packing provisions	Instructions	Special provisions
	3.1.2	2.2	2.2	2.1.1.3	5.2.2	3.3	3.4	3.5.1.2	4.1.4	4.1.4	4.1.10	4.2.5.2 7.3.2	4.2.5.3
(1)	(2)	(3a)	(3b)	(4)	(5)	(6)	(7a)	(7b)	(8)	(9a)	(9b)	(10)	(11)
1170	ETHANOL (ETHYL ALCOHOL) or ETHANOL SOLUTION (ETHYL ALCOHOL SOLUTION)	3	F1	II	3	144 601	1 L	E2	P001 IBC02 R001		MP19	T4	TP1

### Chemical Information

UN No.	1170
NAME	Ethanol (Ethyl Alcohol)
Class	3 = Class 3 Flammable liquid
Classification Code:	F1 = F1 Flammable liquids having a flash-point of or below 60 °C
Packing group	II = Substances presenting medium danger;
Labels	<p>CLASS 3 HAZARD Flammable liquids</p>  <p>(No. 3) Symbol (flame): black or white; Background: red; Figure '3' in bottom corner</p>
Special Provision	144 and 601  Refer to solutions at <24% ethanol V/V and medicines.  <u>This is not relevant for our specific shipment</u>
Limited and Excepted quantities	Max volume as an excepted/limited quantity is 1L or the form (E2): Per inner package 30ml and 1L for the outer package
Hazard ID number (missing from picture)	33 = highly flammable liquid (flash-point below 23 °C)
Limited quantity symbol for air travel	