SOP: Ifakara Ambient Chamber Test (I-ACT)

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Version	Date	Reviewed by	Institution
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2			

Version Control[1]

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Related documents

- I2I Best Practice SOP Library, July 2023 (https://innovationtoimpact.org/)
- Experimental hut preparation and running, I2I-SOP-009
- Specific study procedure recruitment policies
- I-ACT LN test net data collection sheet, Ifakara Health Institute
- I-ACT LN test net rotation sheet, Ifakara Health Institute

[1] Historical versions of SOPs can be found on the I2I website (https://innovationtoimpact.org/)

- Fowler, M.T., et al., The Automatic Classification of Pyriproxyfen-Affected Mosquito Ovaries. Insects, 2021. 12(12)

1. Purpose

The purpose of this document is to provide step-by-step instructions for conducting experiments to measure impact of insecticide treated nets (ITNs) on mosquito mortality, blood feeding and fertility endpoints using the Ifakara Ambient Chamber Test (I-ACT).

2. Background

The Ifakara Ambient Chamber Test [1, 2] is used to measure the bioefficacy of ITNs using mortality, blood feeding inhibition and fertility endpoints. Like the experimental hut, I–ACT makes use of whole nets and human hosts to evaluate bioefficacy of new and field-used ITNs, but the assay is carried out under controlled conditions with laboratory reared mosquitoes. Mosquitoes are released into net chambers within which the test net is hung with a volunteer sleeping beneath. The assay is run overnight, and all mosquitoes are recaptured in the morning. Any mosquito strain or species of interest can be used for testing, and more than one mosquito species can be realised per chamber. However, for the comfort of human volunteers it is recommended that no more than 60 mosquitoes are released per chamber.

Endpoints measured:

Mortality: is measured 24 hours after exposure to the ITN, although for some new chemistries it may be tested after longer periods of time after exposure. A dead mosquito is defined as "Any mosquito that cannot stand or fly in a coordinated manner or that is immobile or shows no sign of life observed at 24 hours (or as per holding time specified in the protocol) after exposure to an insecticide".

Blood feeding: Blood-feeding: a reduction in blood feeding results from when adult female mosquitoes are inhibited from completing the sequence of behaviours that result in a blood

meal. This is a result of interference with the sequence of olfactory and gustatory processes that result in successful host location and/or blood feeding. The impact of an insecticide on mosquito feeding is calculated in I-ACT studies by comparing the proportion of mosquitoes that are fed of all the mosquitoes that were released in that chamber to the proportion of mosquitoes that are fed of all the mosquitoes that were released in the control chamber. Data are checked against an untreated control to ensure that it is the insecticide that is inducing the change in mosquito blood feeding behaviour. The blood feeding rate is used for data analysis and should always be reported.

Blood feeding inhibition (BFI) may also be reported and is the proportion of unfed mosquitoes relative to the untreated control. This can be calculated as follows:

1) Calculate average blood feeding in the control (C) arm.

2) Calculate the blood feeding for each observation for each intervention (T) arm relative to the average blood feeding rate in the control arm using the formula 100x(C-T/C)

3) Calculate the mean blood feeding inhibition (% and 95% CI) from all the observations in each arm.

Effect on fecundity: Reductions in fecundity are decreases in the proportion of females producing viable eggs among the total number of blood-fed adult female mosquitoes. Fecundity is calculated by dissection of mosquitoes to look for viable eggs at a set time (usually 72 hours) after the morning of collecting blood-fed females from an experimental hut. It may also be measured by counting the number of eggs laid by each blood fed mosquito that remained alive long enough to complete egg development and laying. Data are checked against an untreated control to ensure that it is the insecticide that is inducing the change in mosquito fecundity.

3. Materials and equipment

- 1 x Broom per compartment (labelled with compartment number).

- 1x Mouth aspirator per compartment (labelled with volunteer number to minimize transmission of respiratory pathogens). It is advised to use mouth aspirators with HEPA filters to minimize inhalation of chemical residue.

- 1 x Cobweb brush.

- Netted paper cups with cotton wool stoppers (one cup per 20 mosquitoes released or 1 mosquito per 20cm^3 to prevent damage during close confinement.).

- Spare paper cups, rubber bands, netting, and cotton wool for holding mosquitoes.

- Fluorescent marking powder to mark different species if morphologically identical mosquitoes are used in simultaneous releases.

- Small resealable plastic box for transporting mosquitoes. *Note: if mosquitoes are transported for any period this box should be equipped with a data logger and damp towel to maintain humidity and mosquitoes should be provided with sugar solution to minimize handling mortality.*

- Torch light / bulb light.

- Permanent marker and masking tape for labelling paper cups and storage bags.

- Bedsheets.

- Mattress.

- Nets to be tested.

- Bag for each net labelled with test item number. This can be the original packaging or in blinded studies a plain resealable heavy duty plastic bag or foil is used for wrapping test items.

- Data sheet and pen for data recording.

- 10% Sugar solution to give to mosquitoes during the holding period after exposure.

Details about dimensions, materials and how to construct the I-ACT can be found in Appendix 1-4.

4. Procedure

4.1. Recruitment of volunteers will be performed following study specific procedures**.**

4.2. After consent, assign a unique identification (ID) number for each volunteer.

4.3. Screen the volunteer for malaria before enrolment. Weekly malaria screening will be performed according to study needs and recorded on the respective form.

4.4. Volunteers will report to work at least 1hour prior to the experiment start time.

4.5. One volunteer will be allocated to each compartment by the study supervisor following the net rotation sheet as guide.

4.6. Volunteer will be provided with a torch light (if the facility has no power), mouth aspirator and a clean bedsheet.

4.7. Each volunteer will clean their compartment using a broom and cobweb brush to remove predators such as ants and spiders and prepare their sleeping space by placing a clean sheet on the mattress.

4.8. Required nets will be assembled (specific to study protocol and net rotation sheet) from the net storage room.

4.9. Supervisor will hand over each net to the sleeper to correspond with each test chamber following the rotation sheet, 'Ifakara Ambient Chamber LN test net rotation sheet' as guide.

4.10. Each sleeper will hang their given net over the frame in his compartment

4.11. After the sleepers are in their chamber, two or more netted releasing cups containing mosquitoes according to study protocol will be distributed to each sleeper by a supervisor.

4.12. Each sleeper will receive netted "release cups" with specified number (according to study protocol) of nulliparous 3-8-day-old uninfected, actively host seeking mosquitoes.

4.13. Supervisors must check that all the preparations are done correctly in each tunnel, that each participant has all their required equipment in their chamber and check that all interconnecting doors are correctly sealed by means of zips to prevent mosquitoes moving from one test chamber to another.

4.14. Before 21.00 hours, study supervisor performs the final check that all the volunteers are in place and comfortable in all chambers.

4.15. Supervisors must make sure that all the outside doors are closed to ensure no loss of mosquitoes into the wild.

4.16. At 21.00 hours, supervisor will make a loud sound to inform all the volunteers to release the mosquitoes outside of the bednet by gently opening the netted cups while they are situated under the net (Figure 1).

4.17. The supervisor will switch off the light (for the facility with electrical bulb).

4.18. Volunteers must remain in their sleeping space under their ITN until 06.00 hours or as specified in the specific study protocol.

Figure 1: Mosquito release outside the bednet

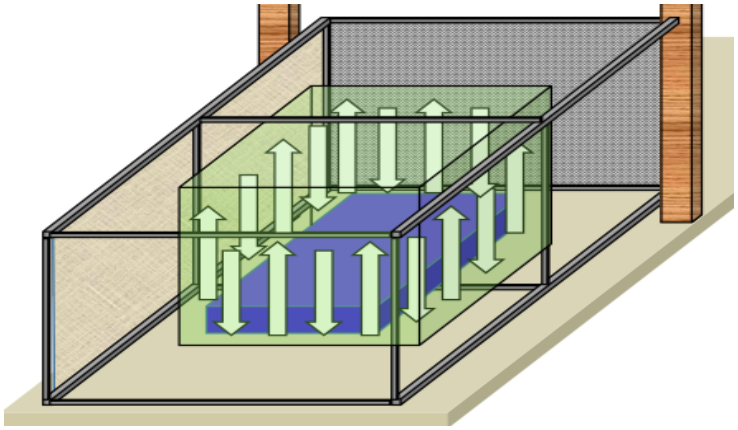


Figure 2: Direction for collection of Mosquitoes inside the bednet

4.19. At 06.00 hours, the volunteers will collect mosquitoes from within their ITN using the mouth aspirator from the bottom right corner of the net in a clockwise direction, working from the bottom up and then down (Figure 2).

4.20. Volunteer will put the collected mosquitoes in the provided paper cup labelled with chamber number and location (the netted releasing cups can be used for convenience).

4.21. Each volunteer will collect knocked down and dead mosquitoes from the floor starting at the right middle side of the sleeping space and working from right to left clockwise until they have checked the entire floor, taking care not to stand on any dead mosquitoes (Figure 3).

4.22. Volunteer will put the collected mosquitoes in the provided paper cup labelled with chamber number and location (the netted releasing cups can be used for convenience).

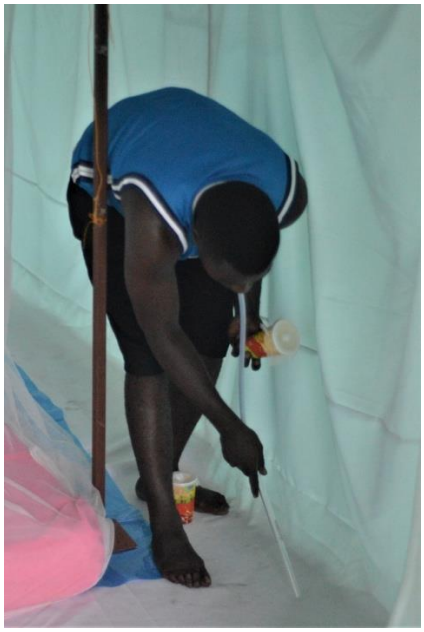


Figure 3: Mosquitoes are collected from the floor working clockwise around the net taking care not to stand on any mosquitoes on the floor.

4.23. After all the mosquitoes have been cleared from the floor, volunteers will start to collect mosquitoes from the floor inside the chamber in the same direction as labelled in Figure 4 from the bottom right corner of the chamber in a clockwise direction, working from the bottom up and then down.

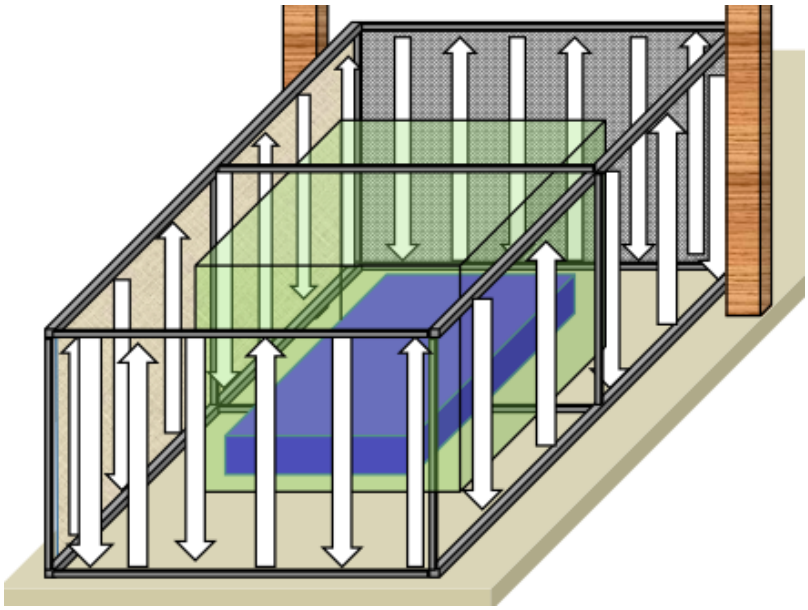


Figure 4 direction of collection of mosquitoes from the walls of the IACT

4.24. Volunteer will put the collected mosquitoes in the provided paper cup labelled with chamber number (same netted releasing cups can be used for convenience).

4.25. The paper cups will be placed in a resealable plastic box for transport to the insectary.

4.26. After the collection of mosquitoes, each volunteer removes the nets from the frame and wraps the net in its respective bag or foil labelled with test item number.

4.27. Volunteers take their sheets from the bed for washing.

4.28. Once all volunteers have completed this procedure, they are free to leave using the double door exit point, ensuring that only one door is open at a time to minimize the chance of any mosquito escape. While leaving, they then hand over the holding cups, net, torch light, siphon and bedsheet in their respective bags to the supervisors and inform supervisors if mosquito numbers collected are incomplete, that is, less than the number released at the start of the test.

4.29. Supervisors estimate recapture of mosquitoes. Each paper cup should contain approximately 20 mosquitoes if full recapture has been obtained.

4.30. In case of incomplete recapture, study technicians will then go inside respective chamber, checking for any remaining mosquitoes that the volunteers may have missed.

4.31. All collection cups are taken to the test area for sorting, scoring, and holding (i.e., monitoring time depend on specific protocol).

4.32. If the test area is far from the I-ACT then mosquitoes should be transported in a box equipped with a data logger and damp towel to maintain humidity and mosquitoes should be provided with sugar solution to minimize handling mortality.

4.33. Technicians should put on disposable gloves during sorting and scoring.

4.34. Mosquitoes are scored as fed alive, unfed alive, fed dead, or unfed dead, and this is recorded in the data collection form.

4.35. If a longer holding period is used continue holding the mosquitoes in the holding insectary at 75% (±20%) relative humidity (RH) and 27±2°C with 10% sugar solution.

4.36. Score the mosquitoes every 24 hours (unless specified otherwise in the study protocol).

4.37. If Control Mortality at 24 hours is greater than 10% or greater than 20% for holding times exceeding 24 hours, disregard the test and repeat it.

4.38. Fertility is estimated by dissection [3] among fed and alive mosquitoes at 72 hours holding time.

4.39. All cups containing mosquitoes are placed into the freezer for 10 minutes to kill the mosquitoes and they are disposed of by placing them in the biohazard waste bin for incineration.

4.40. After each night of test collection, bed sheets must be washed in warm water and dried in the sun and syphons must be decontaminated with ethanol to prevent cross-contamination and to maintain hygiene for the volunteers.

4.41. Empty washing water down a latrine or to a drainage area designated for pesticide disposal.

5. Glossary of terms

For the purposes of this SOP, we classify dead mosquitoes as:

- Mosquitoes that show no movement,
- Mosquitoes that cannot stand or fly in a coordinated manner due to the loss of limbs or wings.

For the purposes of this SOP, we classify alive mosquitoes as:

- Mosquitoes that can stand and fly in a coordinated manner.

For the purposes of this SOP, we classify fed mosquitoes as:

- Mosquitoes that have taken any blood i.e., partially fed or fully fed.

For the purposes of this SOP, we classify fertile mosquitoes as:

- Mosquitoes that have blood fed, survived and developed eggs to Christophers stage V at 72 hours after exposure to a treated ITN.

For the purposes of this SOP, we classify infertile mosquitoes as:

- Mosquitoes that have blood fed, survived and developed eggs to Christophers stage I to III at 72 hours after exposure to an insecticide.

6. Abbreviations

BFI	Blood feeding inhibition
C	Control
I2I	Innovation to Impact

I-ACT	Ifakara Ambient Chamber Test
ID	Identification
IHI	Ifakara Health Institute
ITN	Insecticide-treated nets
LSTM	Liverpool School of Tropical Medicine
RH	Relative humidity
SOP	Standard operating procedure
T	Treatment

7. References

1. Kibondo, U.A., et al., *Comparison of bioassays for the evaluation of chlorfenapyr: evaluation of an alphacypermethrin Insecticide Treated Net (ITN) Interceptor and alphacypermethrin chlorfenapyr ITN Interceptor G2 by WHO Cone and Tunnel test, Ifakara Ambient Chamber Test and Experimental Hut test in Tanzania* Parasites and Vectors, 2021. **15**: p. 124.

2. Massue, D.J., et al., *Comparing the new Ifakara Ambient Chamber Test with WHO cone and tunnel tests for bioefficacy and non-inferiority testing of insecticide-treated nets.* Malaria Journal, 2019. **18**(1): p. 153.

3. Fowler, M.T., et al., *The Automatic Classification of Pyriproxyfen-Affected Mosquito Ovaries.* Insects, 2021. **12**(12).

Project Name |_____| Study serial: |_|_|_|_| Study director (Initials): |___|___|___|

Date of test (dd/mm/yyyy): |_|_|/|_|_|/|_|_| Age of mosquitoes in days: |___| - |___|

Temperature |_|_| C Humidity |_|_|%

Start time (hh:mm): |_|_|:|_|_| End time (hh:mm) |_|_|:|_|_|

Check time period of observation

12 hour |__| 24 hour |__| 48 hour |__| 72 hour |__|

96 hour |__| 120 hour |__| 144 hour |__| 168 hour |__|

Scored by (Initials): |___|___| Entry by (Initials): |___|___|

Item code	Volunteer number	Test strain number	Total Exposed	Compartment number	BF alive	BF dead	UnF alive	UnF dead	Total recaptured

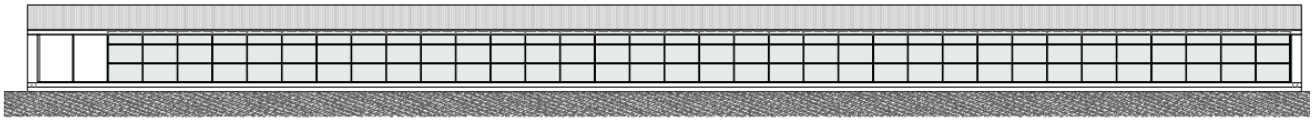
Where relevant: IN-INSIDE NET into which mosquitoes are released; OUT: Outside net but within tunnel compartment

Verified by study director/coordinator:_____ Signature: _____ Date (dd/mm/yy): |_|_|/|_|_|/|_|_|

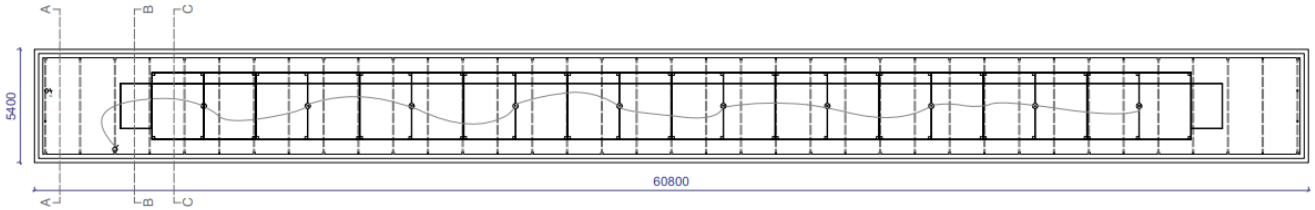
Test strain number: e.g. *An. gambiae s.s.* (Ifa) - 1, *An. arabiensis* (King) - 2, *An. gambiae s.s.* (Kis) - 3, *An. gambiae s.s.* (KDR) - 5, *Aedes aegypti* (Bag) - 6, *Culex quinquefasciatus* (Bag) - 7, *An. funestus* (Moz) - 8, *Ae aegypti* (Kig) 9, *An. arabiensis* (Sud)

Appendix

1. I-ACT Floorplan



Elevation



Groundfloor

Light bulb
Switch
Socket

legend

IFAKARA HEALTH INSTITUTE
research | training | services
BAGAMOYO - Kingani Research Center

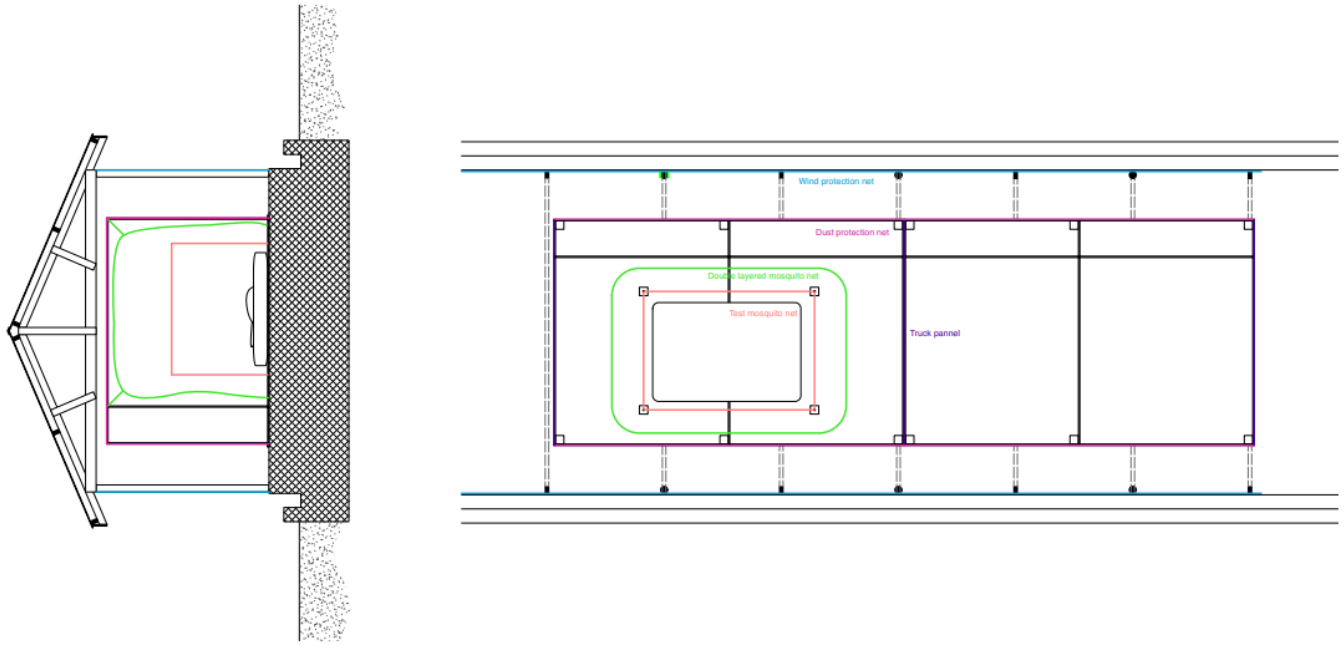
CSRS Mosquito tunel
groundfloor plan
project name

1:150
dinA3
scale and size

9/11/2022 for build. perm.
date and modification

N
S. Pfammatter
P. Sasse
authors

2. I-ACT material details



Truck panel
Dust protection net
Wind protection net
Double layered mosquito net
Test mosquito net

legend

IFAKARA HEALTH INSTITUTE
research | training | services
BAGAMOYO - Kingani Research Center

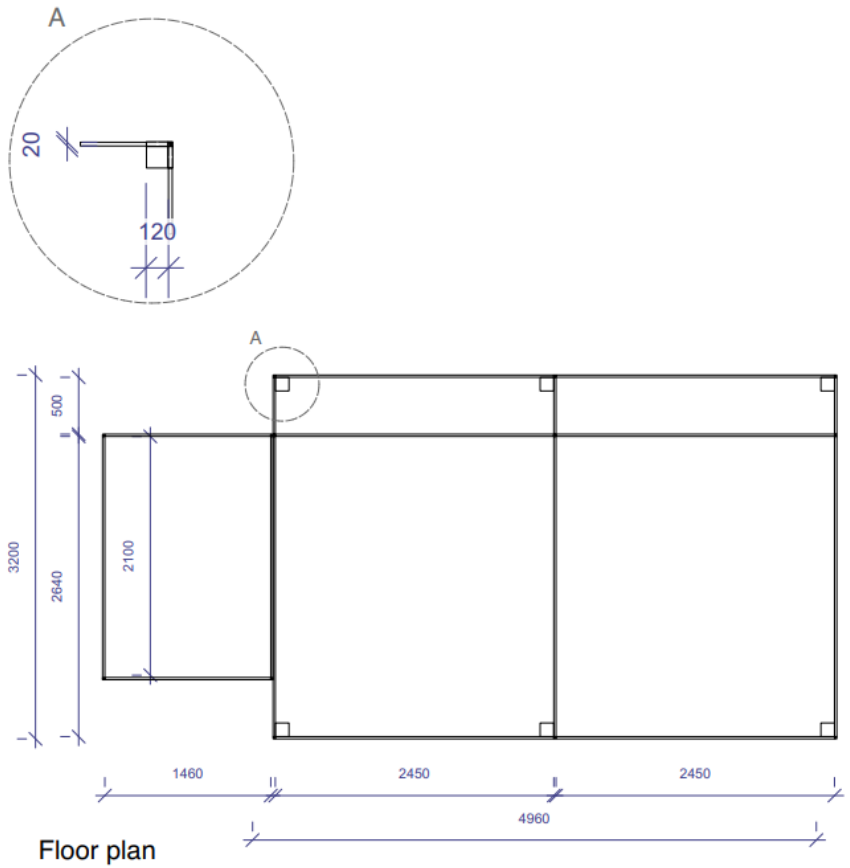
CSRS Mosquito tunel
detail materials
project name

1:50
dinA3
scale and size

9/11/2022 for build. perm.
date and modification

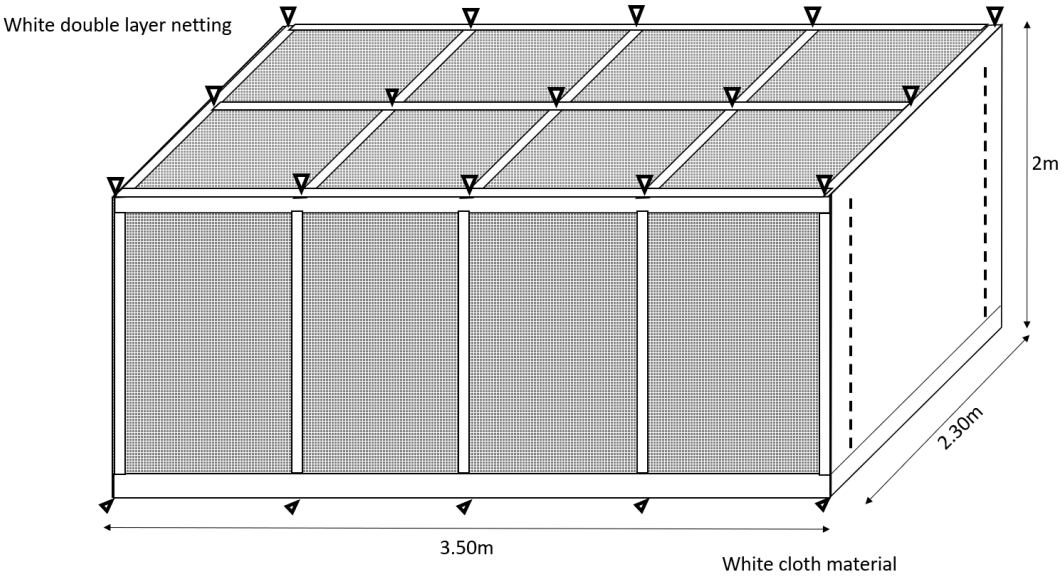
N
S. Pfammatter
P. Sasse
authors

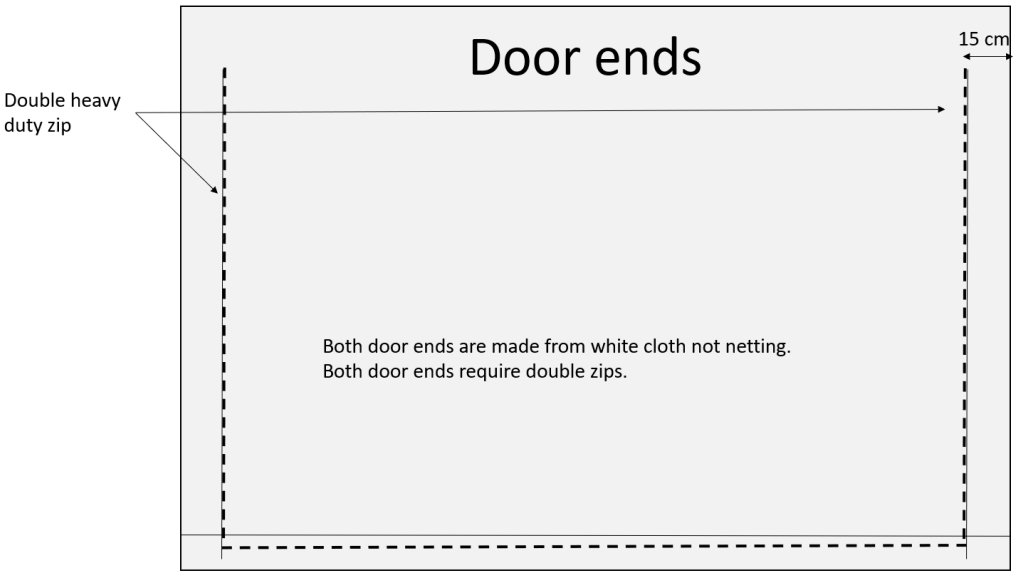
3. I-ACT construction floorplan



legend	IFAKARA HEALTH INSTITUTE research | training | services BAGAMOYO - Kingani Research Center	CSRS Mosquito tunel metall construction project name	1:50 dinA3 scale and size	9/11/2022 for build. perm. date and modification	N S. Pfammatter P. Sasse authors

4. Instructions on making individual cloth and net chambers





- To be sewn as per sample to the dimensions shown in figure.
- Netting to be untreated.
- White heavy-duty cloth to be used as per sample.
- All seams sewn on the outside.
- Good quality heavy duty double zips to be used.
- Order to come with protective bag for each unit.
- All 10 units identical.