I2I Landscaping exercise

Ifakara Ambient Chamber Test (I-ACT)

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## Acronym List

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<th>Acronym</th>
<th>Description</th>
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<tr>
<td>I-ACT</td>
<td>Ifakara Ambient Chamber Test</td>
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<tr>
<td>IHI</td>
<td>Ifakara Health Institute</td>
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<tr>
<td>ITNs</td>
<td>Insecticide-treated nets</td>
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<td>SOPs</td>
<td>Standard operating procedures</td>
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<td>WHO</td>
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## Summary

| Aim and key questions addressed | - Quantifies practical protection of nets from host-seeking mosquitoes (in a closed, climate-controlled environment)  
- Used to assess protective efficacy of bed nets sampled from the field as part of longitudinal durability evaluations |
| Context                          | - Laboratory |
| Test item                        | - Insecticide-treated nets (ITNs) |
| Mosquito population              | - Laboratory reared |
| Number of mosquitoes per replicate | 30 |
| Endpoints measured              | - Blood-feeding inhibition  
- 24-hour mortality (delayed mortality for newer active ingredients) |
| Exposure time                    | - Overnight |
| Holding time                     | - See relevant protocol for active ingredient tested |
| Indicative of personal protection | - Yes |
| Suitable chemistries             | - Chemistries applied to ITNs |
| Appropriate controls             | - Negative control: untreated netting  
- Positive control: new, unused example of the net under evaluation |
<p>| Relevant stage of production pipeline | - Durability assessment |</p>
<table>
<thead>
<tr>
<th>Characterisation of output</th>
<th>- Endpoints for pyrethroid nets are well defined. Endpoints have been defined for nets with different active ingredients, however these need to be validated</th>
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<tr>
<td>Accessibility</td>
<td>- Key challenges are the recruitment of volunteers, collecting mosquitoes at end of assay, additional need to clean testing room between assays, space requirement to build test chambers</td>
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<td>Cost</td>
<td>- Cost of materials to build testing chamber and volunteer costs</td>
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| Level of validation and characterisation of outputs | - Published comparisons with cone and tunnel tests are available  
- However, methodology not standardised by WHO and test needs to be validated |
| Outstanding questions, gaps and priorities | - This method requires validation |
Overview

The Ifakara Ambient Chamber Test (I-ACT) is a new standardised semi-field assay (Massue et al., 2019) used to evaluate the bioefficacy of whole insecticide-treated nets (ITNs).

In brief, the I-ACT is a 50m long, 3m wide and 2.1m high steel tube frame construction (Figure 1) covered by durable UV resistant polyurethane coated netting with an overlaid polyurethane sheet to minimise wind so that bioassays are conducted in still air. The tunnel sits beneath a wooden frame supporting a steel roof to allow experiments to be performed in all weather conditions. The netted tunnel is divided into ten individual test chambers with interconnecting doors that are sealed with zips and Velcro to prevent mosquitoes moving between chambers. Each compartment contains a white netted chamber 5m long, 2m wide and 2m high in which the ITN is hung from a frame with a human volunteer sleeping underneath. At each end of the tunnel is an additional double door module to ensure no loss of laboratory-reared mosquitoes into the wild. Mosquitoes are released from holding cages within each chamber by the human volunteer. After the allotted experimental time all mosquitoes within each of the compartments are recovered by mouth aspiration (those within the ITN) and by battery powered Prokopack aspirators (outside the net but within the compartment). The allows whole ITNs to be tested in a controlled ambient chamber with a human host sleeping beneath to measure the protective efficacy of the net, examining both personal protection through feeding inhibition and community protection by mosquito mortality.
Define Accepted Methodologies

Are there existing standard SOPs/Guidelines detailing methodologies?

- Ifakara Ambient Chamber Test, I2I-SOP-036
- (Massue et al., 2019) Additional file 2 ‘A standardised operating procedure for conducting experiments to measure feeding inhibition and mortality of different net products using the I-ACT’.
Are these sufficiently detailed?

The SOP is sufficiently detailed and includes information on how to perform the assay accurately, as well as additional information on how to build an I-ACT.

Do these methods require specialised/non-standardised equipment and/or training?

This methodology requires access to space and equipment to build the I-ACT. Materials are not costly, but enough space is needed for 10 chambers to be built.

Are there issues with the methods or their interpretation?

There are no known issues with interpretation.

What AIs or combinations of AIs have the tests been used for?

Insecticide-treated nets: Olyset Net (permethrin), PermaNet 2.0 (deltamethrin), Netprotect (deltamethrin), Tsara soft (deltamethrin), Interceptor G1 (alphacypermethrin), Interceptor G2 (alphacypermethrin plus chlorfenapyr).

Are they validated, for which AIs/entomological effects, and to what extent?

A study by Massue (Massue et al., 2019) evaluated the bioefficacy of whole ITNs that were returned from the field in a longitudinal durability study. This study measured the bioefficacy of used (field-aged) ITNs using the I-ACT assay and standard WHO durability testing bioassays (cone and tunnel tests). The proportion of nets passing WHO criteria by standard methods and the I-ACT was compared, with the aim of demonstrating the utility of this assay for measuring
the bioefficacy of different ITN products and to explore its use for non-inferiority testing of new products. The influence of testing modality on bioefficacy for the evaluation of next generation dual-active ingredient nets has also been investigated (Kibondo et al., 2022). This study evaluated the performance of nets measured by four bioassay types (WHO cone test, WHO tunnel test, I-ACT and WHO experimental huts) with results showing that both free-flying assays (I-ACT and tunnel test) consistently measured similarly, and both predicted the results of experimental hut tests.

There are currently three more Ifakara Ambient Chambers being built for method validation.

What inputs need to be characterised? e.g., samples, mosquitoes, equipment

- Mosquito number
- Mosquito strain characterisation – laboratory reared mosquitoes should be characterised (Lees et al., 2022)

Are endpoints clearly defined and appropriate? Who were they defined by?

Endpoints of mosquito mortality and blood-feeding inhibition are defined in the SOP.

Are their supporting SOPs? e.g., cleaning SOPs, mosquito rearing SOPs required

- I-ACT LN test net rotation sheet, IHI
- Experimental hut preparation and running, I”I-SOP-009

Define Current Use Practices
Does everybody use the same SOP?

There is currently only one SOP to follow.

Are there differences of interpretation of the method?

There are no reported differences in method interpretation to date.

Are there results obtained largely consistent between studies?

N/A

Is further development, refinement or validation of the method required? Based on priority, significance, and relevance of method.

External validation is currently being conducted in multiple sites.

Identify Potential Sources of Variation

What are the sources of variability in the method and are their means to minimise or characterise these?

- Mosquito age and species: Mosquitoes used for the I-ACT are reared and released into individual testing chambers. This allows mosquitoes to be reared under tight rearing
protocols to ensure uniformity across colonies. Multiple different species can be tested per night as each testing chamber is individually sealed to prevent mosquito escape.

- Temperature and humidity conditions are those of the outside environment, and so will be uniform across all testing chambers on any given testing night.

Does current method/s need to be adapted for new active ingredients/MoA/types of tool?

The I-ACT may prove useful in evaluating new products that function through either mortality or feeding inhibition.

Are new methods required? Identify areas where current method/s are not suitable or sufficient.

N/A

Gaps in biological or other understanding that hinder method development or validation

N/A

Prioritisation – is there an issue that needs to be addressed, what specifics, how urgent is the need?

The I-ACT is currently being validated in multiple external testing sites. The results from these studies will address the suitability of the I-ACT to be used by different research groups, in different locations for different net types.
References


