I2I Landscaping Exercise

WHO Wire ball bioassay

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Acronym List

I2I	Innovation to Impact
ITN	Insecticide-treated net
LLIN	Long-lasting insecticidal net
SOPs	Standard operating procedures
WHO	World Health Organisation
WHOPES	World Health Organisation Pesticide Evaluation Scheme
WHO PQ/VCP	World Health Organization Prequalification / Vector Control Products

Summary

Please note: WHO PQ/VCP Implementation Guidance documents state that 'the wire ball test has not been robustly validated and there is little supporting evidence for it's use as a standardised bioassay for ITN assessment'.

Aim and key questions addressed	- Used to benchmark bioefficacy of a net sample against a well characterised mosquito strain by forcing mosquito into proximity with a net - Comparing net samples sampled from the field at timepoints after distribution can detect longitudinal changes in bioefficacy - Can be used to quantify the time taken to knock-down majority of susceptible mosquitoes
Context	- Laboratory
Test item	- Insecticide-treated nets (ITNs)
Mosquito population	- Laboratory reared
Number of mosquitoes per replicate	- 11
Endpoints measured	- Time to knock-down - 1 hour knock-down - 24-hour mortality
Exposure time	- 3 minutes
Holding time	- See relevant protocol for active ingredient tested
Indicative of personal protection	- No

Suitable chemistries	- Chemistries applied to treated nets
Appropriate controls	- Negative control: untreated netting (ideally equivalent fabric to test item) - Positive control: new, unused examples of relevant ITN product
Relevant stage of production pipeline	- Product development - Durability assessment
Characterisation of output	- Not complete
Accessibility	- Materials and set-up in line with cone test but in practice more difficult to remove mosquitoes at end of assay
Cost	- Low
Level of validation and characterisation of outputs	- Impact of the following variables not well described: ○ Difference between cube and sphere ○ Mosquito number per assay ○ Mosquito age ○ Number of biological replicates per net piece ○ Number of net cuttings tested per net ○ Location on net where netting samples are cut from
Outstanding questions, gaps and priorities	- Validation and characterisation of outputs
Key references, related SOPs, guidelines and publications	- WHO., (2016) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization. https://doi.org/10.1007/978-3-642-10565-4

Overview

The World Health Organisation (WHO) wire ball bioassay is a method for exposing mosquitoes to a piece of netting insecticide-treated net (ITN) or long-lasting insecticidal net (LLIN). The netting sample is wrapped around a metal frame, creating a fully enclosed area (Figure 1). The purpose of this technique is to investigate the bioefficacy of a net that has been collected from the field in comparison to a new net of the same brand. By comparing used nets with new nets, longitudinal changes in bioefficacy over time can be detected. However, due to the complex interaction between a mosquito and a bed net in real use, bioefficacy in the wire ball is not intended to be representative of epidemiological protection.

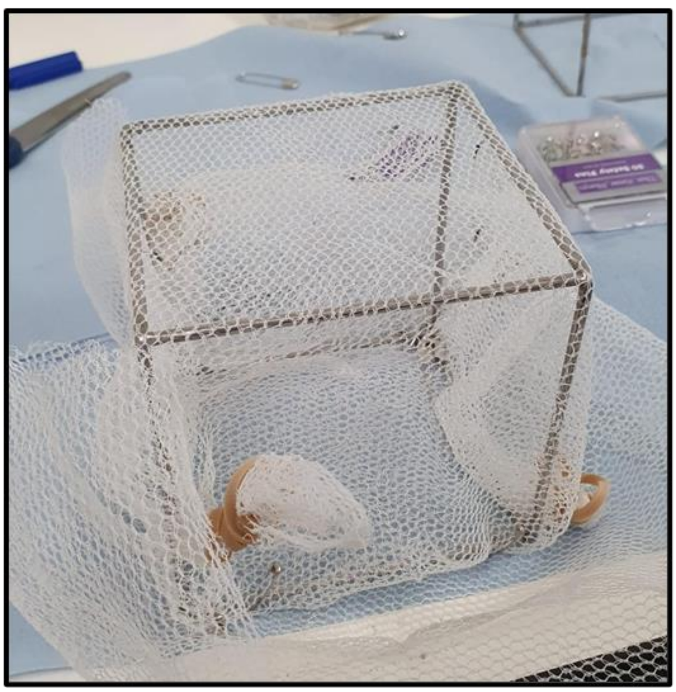

Figure 1. Image of cube variant of WHO wire ball assay in use. The image shows netting material wrapped around the metal cube frame and secured in place with elastic bands.

By surrounding the frame with the netting, it is assumed that the mosquitoes released inside cannot avoid contact with the net by flying away from the net surface. The current WHO methodology for the wire ball assay describes two different acceptable frames which can be used to affix the net; either a 15 cm cube or a sphere made up of two intersecting circles 15 cm in diameter (WHO, 2006). For evaluating the residual activity of a net, it is indicated that 10 mosquitoes are aspirated into the internal space through a 'sleeve' and left inside for three minutes before being removed through the same entrance hole. Mosquitoes are then placed into a holding container and assessed for 24-hour mortality. The methods also describe the use of this assay for assessing median knockdown time when high mortality rates are observed, with the operator visually inspecting the activity of 11 mosquitoes aspirated inside to determine which have been knocked down. However, it does not specify the thresholds for 'high' mortality, and it is unclear why 10 mosquitoes are assessed for mortality, while 11 were used for time to knockdown. It should be noted that the description of wire ball methods in the WHO technical document is minimal (WHO, 2006).

Define Accepted Methodologies

Outcomes measured

Outcomes explicitly described in methods:

- 24-hour mortality
- Time-to-knockdown

Outcomes readily inherited from cone methods (but not described in wire ball standard operating procedure (SOP):

- 1hr knockdown
- 72-hour mortality
- Longevity

Current use practices

In the initial documentation, the WHO wire ball assay was only explicitly outlined for use in Phase II and III World Health Organisation Pesticide Evaluation Scheme (WHOPES) studies.

However, it can assess the same outcomes as the WHO cone bioassay thus would be useful in early net development studies and later quality control.

- The intersecting circle setup appears to be used much more widely than the cube method (9/10 studies which specified (Table 1)
- 'Time to knockdown' is the most frequent application in existing literature.
- It is also used in a small number of studies to ensure net contact before investigating outcomes other than bioefficacy, such as the impact of LLIN exposure on *P. falciparum* development (Kristan et al., 2016) and quantifying insecticide uptake (Kristan et al., 2020).
- Used as a reliable method of exposing mosquitoes to net (as opposed to the WHO Cone in which the mosquito may avoid contact (Owusu & Müller, 2016).

Potential sources of variation

- Use of frame set up (cube or sphere)
- Number of mosquitoes per assay (though consistently 10 or 11 in literature): In the 2006 guidelines, an explanation of calculating time-to-knockdown describes a practical example where six out of 11 mosquitoes are knocked down. Subsequent studies using the wire ball appear to have taken this example as direction to use 11 mosquitoes per assay, but it is not explicitly clear if this was intended as a directive.
- Location on ITN where sample is cut from: Potential point of contention between manufacturers as some products have different insecticides profiles on different net surfaces.
- Number of replicates per sampled ITN and repeat measurements per net piece.
- Technique for affixing netting around frame: Lack of consensus SOP leaves room for interpretation and variation on how a net should be affixed to the frame.
- Collecting net samples for use in wire ball assays requires forethought as net pieces must be large enough to fit around the frame (a 30 cm x 30 cm net cutting will not fully surround a correctly sized cube), and this is not specified in current guidelines.

- Due to the methodology, time taken to recollect mosquitoes when removing them from the wire ball frame varies substantially between users. The large internal volume of the cube or sphere can make it difficult to quickly remove mosquitoes at the end of the assay with a mouth aspirator. While this can be addressed by using a mechanical aspirator, the addition of equipment decreases the accessibility of the assay. The assay can still be performed with a mouth aspirator however this introduces a potential bias into the experiment as this 'time to remove' potentially results in additional exposure above three minutes as there is a large interval volume for mosquitoes to move around in.

Outstanding questions & gaps

- Is there a practical difference in outcomes between the frame used (cube or sphere)? Markedly different interval volume (sphere = 1767cm^2, cube = 3375cm^2)
- Is the interval volume relevant for outcomes (e.g., would a smaller cube, which could be more practical for mosquito removal, result in a different outcome)?
- What is the relationship in outcomes between the same net sample tested in the cone and wire ball (particularly with excito-repellent products)?
- Poor understanding of how the excito-repellency of a compound and resulting movement within the assay impacts outcomes.

Level of validation

The effect of the following outcomes of the WHO wire ball assay are poorly described:

- Use of sphere or cube
- Mosquito age
- Exposure time
- Number of mosquitoes in each exposure
- Number of mosquitoes per individual net sample/cutting

- Number of net cuttings per whole ITN
    - Location on the net where these cuttings are taken from (e.g., top, side)
- Appropriate climactic conditions for performing assays
    - While there is an agreed standard (26 ± 2 °C and 70 ± 10% relative humidity) how well this represents practical use is a point of contention.

Additionally, the use of pyrethroid-resistant mosquitoes raises several key issues (though these issues are part of an ongoing discussion of the use of pyrethroid-resistant mosquitoes in bioassays).

- What is the definition of 'resistant' when demonstrating efficacy against resistant mosquitoes in the wire ball?
    - Is the standard WHO tube definition used (WHO, 2016)?
    - Does the strain need to be site-specific to the country nets were sampled from?

Conclusion

The WHO wire ball assay is somewhat poorly defined method for assessing the bioefficacy of ITNs and LLINs. The method requires standardisation, with a resolution to the unusual latitude of allowing the user to choose between two different physical set-ups (ball or cube) with a large disparity in volume. However, given the need for bioassay methods that prevent mosquitoes from avoiding the net (due to the need to assess bioefficacy of products with excito-repellent properties) the wire ball has potential to be a mainstream tool in net durability studies.

There is a need for a detailed and unambiguous SOP for conducting the WHO wire ball, with an argument to be made for choosing the wire or cube as the definite method. Additionally, there is a lack of validation of the technique, with a need to determine the optimal number of mosquitoes and optimal exposure time.

Next steps

Development of a consensus SOP that establishes unambiguous methodology.

References & key documents

Kristan, M., Lines, J., & Kaur, H. (2020). Determination of the amount of insecticide picked up by mosquitoes from treated nets. *BioRxiv*, 2020.08.07.241224. https://doi.org/10.1101/2020.08.07.241224

Kristan, M., Lines, J., Nuwa, A., Ntege, C., Meek, S. R., & Abeku, T. A. (2016). Exposure to deltamethrin affects development of Plasmodium falciparum inside wild pyrethroid resistant Anopheles gambiae s.s. mosquitoes in Uganda. *Parasites and Vectors*. https://doi.org/10.1186/s13071-016-1384-x

Owusu, H. F., & Müller, P. (2016). How important is the angle of tilt in the WHO cone bioassay? *Malaria Journal*, *15*(1), 1–10. https://doi.org/10.1186/S12936-016-1303-9/FIGURES/6

WHO. (2006). *Guidelines for testing adulticides for indoor residual spraying and treatment of mosquito nets*. https://doi.org/WHO/CDS/NTD/WHOPES/GCDPP/2006.3

WHO. (2016). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. World Health Organization. https://doi.org/10.1007/978-3-642-10565-4

Table 1. Summary of published studies which utilise the WHO wire ball assay with description on frame used and outcomes measured.

Author	Year	Title	Frame shape	Outcomes assessed
WHO	1996	Report of the WHO informal consultation on the evaluation and testing of insecticides	NA	NA
Curtis et al.	1998	A comparison of use of a pyrethroid either for house spraying or for bednet treatment against malaria vectors	Ball	Time-to-knockdown
Hodjati et al.	2003	Irritant effect, prevention of blood feeding and toxicity of nets impregnated with different pyrethroids on *An.stephensi*	Ball	1hr knockdown and 24hr mortality
Yates et al.	2005	Evaluation of KO-Tab 1-2-3®: a wash-resistant 'dip-it-yourself' insecticide formulation for long-lasting treatment of mosquito nets	Not specified	Time-to-knockdown
Graham et al.	2005	Multi-country field trials comparing wash-resistance of PermaNet™ and conventional insecticide-treated nets against anopheline and culicine mosquitoes.	Not specified	Time-to-knockdown
Maxwell et al.	2006	Test of Olyset nets by bioassay and in experimental huts	Ball	1hr knockdown and 24hr mortality (comparison of cone and wire-ball)
WHO	2006	Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets	NA	NA
N'Guessan et al.	2007	Chlorfenapyr: A pyrrole insecticide for the control of pyrethroid or DDT resistant Anopheles gambiae (Diptera: Culicidae) mosquitoes	Not specified	1hr knockdown and 24hr mortality (comparison of cone and wire-ball)
Muller et al.	2008	Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in *Anopheles arabiensis* sampled from an area of cotton fields in Northern Cameroon	Cube	Time-to-knockdown

Okumu et al.	**2012**	**Implications of bio-efficacy and persistence of insecticides when indoor residual spraying and long-lasting insecticide nets are combined for malaria prevention**	**Ball**	**1hr knockdown and 24hr mortality (comparison of cone andire-ball)**
Chanda et al.	**2013**	**Field evaluation of KO-Tab 1-2-3® long lasting insecticidal net performance in Milenge, Zambia**	**Ball**	**Time-to-knockdown**
Okia et al.	**2013**	**Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of *Anopheles gambiae s.s*. from different malaria transmission zones in Uganda**	**Ball**	**Time-to-knockdown**
Oxborough et al.	**2015**	**A new class of insecticide for malaria vector control: evaluation of mosquito nets treated singly with indoxacarb (oxadiazine) or with a pyrethroid mixture against *Anopheles gambiae* and *Culex quinquefasciatus***	**Not specified**	**1hr knockdown and 24hr mortality**
Kristan et al.	**2016**	**Exposure to deltamethrin affects development of *Plasmodium falciparum* inside wild pyrethroid resistant *Anopheles gambiae s.s.* mosquitoes in Uganda**	**Not specified**	**Sublethal effects**
Angela Hughes PhD thesis	**2018**	**Impact of Exposure to Long Lasting Insecticide Treated Nets on Mosquito Survival and Behaviour at the Net Interface in Insecticide Susceptible and Resistant Strains of the Afrotropical Anopheles mosquito**	**Ball**	**1hr knockdown and 24hr mortality**
Kristan et al.	**2018**	**Effect of environmental variables and kdr resistance genotype on survival probability and infection rates in *Anopheles gambiae (s.s.)***	**Ball**	**24hr mortality**
Tan et al.	**2019**	**The polymorphism and geographical distribution of knockdown resistance of adult *Anopheles sinensis* populations in eastern China**	**Ball**	**Time-to-knockdown**
Kristan et al.	**2020**	**Determination of the amount of insecticide picked up by mosquitoes from treated net**	**Not specified**	**Insecticide uptake**

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