SOP: WHO wire ball bioassay

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Version Control[1]

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Related documents

- I2I Best Practice SOP Library, 30 October 2020 (https://innovationtoimpact.org/)

1. Purpose

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 its use as a standardised bioassay for ITN assessment'.**

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).

2. Background

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,

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

bioefficacy in the wire ball is not intended to be representative of epidemiological protection.

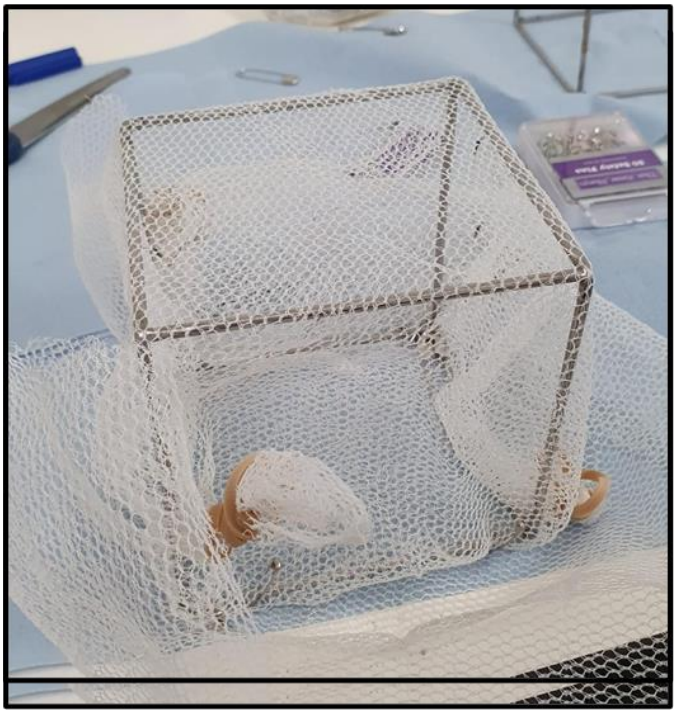

Figure 1.. Image of cube variant of WHO wire ball assay in use. The image shows netting material wrapped around the metal cube frame and secured in place with elastic bands.

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). To evaluate the residual activity of a net, it is indicated that 11 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. It should be noted that the description of wire ball methods in the WHO technical document is minimal (WHO, 2006).

The definitions of mortality and knock-down are those recommended by WHO (WHO, 2013). Mosquitoes are considered to be alive if they can both stand upright and fly in a coordinated manner. Mosquitoes that are moribund or dead are classified and recorded as knocked down at 60 minutes and as dead at 24 hours. A mosquito is moribund if it cannot stand (e.g. has one or two legs), cannot fly in a coordinated manner or takes off briefly but falls immediately. A mosquito is dead if it is immobile, cannot stand or shows no signs of life.

3. Materials and equipment

a. Preparation of test room
    i. Paper cups
    ii. Marker pen
    iii. 10% bleach
    iv. Bench guard
    v. Masking tape
b. Pre-exposure period
    i. Aspirator
    ii. Clock/ Timer
    iii. Environmental conditions log sheet
c. Preparation for assay
    i. Gloves
    ii. Lab coat
    iii. Untreated net pieces
    iv. Treated net pieces (Net pieces to be tested should be packed individually in foil).
    v. Rubber bands
    vi. Marker pen/label
    vii. Aspirators
    viii. Wire ball frame (either a cubical frame of 15 x 15 x 15cm, or two intersecting circles of 15cm in diameter)
    ix. Stopwatch/timer

x. Masking tape
xi. Cup racks
xii. Aluminum foil
xiii. Environmental conditions log sheet
xiv. Data log sheet

d. Exposure
    i. 10% sucrose soaked cotton wool
e. Post- exposure
    i. +4°C refrigerator
    ii. Decontamination spray (according to protocol, active ingredient)
    iii. Biohazard bag

4. Procedure

1. **Preparation of test room and materials**
    a. Label paper cups with the protocol code, date of exposure, test item, exposure length, and replicate number
    b. Ensure the wire frames, aspirators and bench area have all been cleaned in 10% bleach and rinsed twice with clean water.
    c. Place a clean bench guard on top of the bench, and fix with masking tape.

2. **Acclimation (pre-exposure) period**
    a. Remove the glucose-soaked cotton wool from the mosquito cage for at least one hour before starting all testing process.
    b. Remove or reduce, if possible, males from the cage to be used for testing.
    c. Aspirate five female mosquitoes into each paper cup. Choose female mosquitoes that are fit, appropriately sized, and able to fly consistently. Do not choose mosquitoes that are small, missing one leg or wings, or that are unable to fly in a coordinated manner.
    d. Allow mosquitoes to acclimatize for one hour.
    e. Record the temperature, humidity, logger ID number, and time acclimation started on the form.

3. **Preparation for the wire ball bioassay**
    a. Put on gloves and lab coat.
    b. Cover the paper cups with the untreated net.
    c. Label paper cups (or disposable cups) with the protocol code, date of exposure, test item, exposure length, and replicate number.
    d. Prepare the wire frames on the bench.
    e. Prepare all the net pieces. Starting with the control, unwrap the net piece from the foil and position the net piece around the frame, leaving a sleeve to aspirate mosquitoes in through.
    f. Record the temperature, humidity, logger ID, and time acclimation period ended on the form.

4. **Exposure**
    a. Aspirate eleven, non-blood-fed female mosquitoes from a cup into the negative control Ensure that correctly labelled aspirator is used to avoid cross-contamination.
    b. Once all of mosquitoes are in the first wire ball, start the timer.
    c. Wait for one minute and aspirate eleven mosquitoes into the next wire ball.
    d. Repeat this procedure until mosquitoes have been introduced into all wire balls, changing gloves and aspirator if exposing mosquitoes to different treatments.
    e. Three minutes after mosquitoes have been introduced into the first wire ball, aspirate the mosquitoes back into the labelled paper cup (or disposable cup).
    f. Write the time at which the exposure was ended for each cone and read the knock down 60 minutes later (see section 7 for knockdown assessment criteria).
    g. Continue until all the samples have been tested.
    h. Place glucose-soaked wool on top of the cups after recording knockdown and place in cup racks.

5. **Post Exposure**

a. Return net pieces to corresponding aluminium foil, wrap and put in the +4°C refrigerator or designated storage following testing.
b. Decontaminate all insecticide-contaminated material according to the relevant SOP.
c. Dispose of bench guard in a biohazard bag.
d. Store mosquitoes in either silica beads or in the freezer if future analysis (such as wing measurements) are required.

5. Data collection

Collection and reporting of data

Data analysis

– If knockdown and mortality in the negative control is < 5%

$$\text{KD60 (\%)} = \frac{\text{Total number of knocked down mosquitoes}}{\text{Total number of mosquitoes tested}} \times 100$$

$$\text{Observed mortality (\%)} = \frac{\text{Total number of dead mosquitoes}}{\text{Total number of mosquitoes tested}} \times 100$$

– If knockdown and mortality in the negative control is >5% but <10% correct using Abbott's formula. Control mortalities of <5% require no correction.

Abbott's formula:

$$\text{Corrected mortality} = \frac{(\%\text{ observed mortality} - \%\text{ control mortality})}{(100 - \%\text{ control mortality})} \times 100$$

– When mortality is >10%, the results should be discarded except for when there is delayed mortality when results may be retained up until the time at which control mortality exceeds 10%.
– Ensure the following data is recorded in the data collection sheets.
    a. Date of exposure
    b. Protocol followed

c. Test item ID
d. Test system species and strain
e. Age of mosquito
f. Fed status
g. Exposure conditions
    i. Day or night
    ii. Time length of test
h. Outcome measures
    i. Knock down time
    ii. Mortlity time
i. Environmental conditions for
    i. Acclimation
        1. Start and End
            a. Data logger ID number
            b. Temperature (C)
            c. Humidity (%)
            d. Time
            e. Initials of staff
    ii. Exposure
        1. Start and End
            a. Data logger ID number
            b. Temperature (C)
            c. Humidity (%)
            d. Time
            e. Initials of staff
    iii. Post-exposure holding
        1. 60 minutes, 24 hours, etc
            a. Data logger ID number
            b. Temperature (C)
            c. Humidity (%)
            d. Time
            e. Initials of staff

j. Scoring
    i. Test item code
    ii. Start time of exposure
    iii. Insecticide code
    iv. Replicate number
    v. Number of mosquitoes tested
    vi. 60 minute KD
    vii. 24 hour mortality (up to 72 hour mortality if needed)

6. Deviations from standard protocol

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

- 1hr knockdown
- 72-hour mortality
- Longevity

7. Glossary of terms

6. Recording knock down and mortality

a. Knock down. Mosquitoes are scored as being alive if they can both stand upright and fly in a coordinated manner. Mosquitoes that are moribund or dead are classified and recorded as knocked down at 60 minutes.
b. Mortality. Mosquitoes that are moribund or dead are classified and recorded as dead at 24 hours (or later time point for treatments giving delayed mortality).
c. A mosquito is moribund if it cannot stand (e.g. has one or two legs), cannot fly in a coordinated manner or takes off briefly but falls immediately. A mosquito is dead if it is immobile, cannot stand or shows no sign of life.

8. Acronyms

I2I	Innovation to Impact
ID	Identification
ITN	Insecticide treated net
KD	Knock down
LLIN	Long-lasting insecticidal net
LSTM	Liverpool School of Tropical Medicine
SOP	Standard operating procedure
WHO	World Health Organization

9. References

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. (2013). Guidelines for laboratory and field-testing of long-lasting insecticidal nets. In *WHO/HTM/NTD/WHOPES/20131*. World Health Organization.