



Noise bioassays

Investigating the level of variation within standard bioassays

Dr Kath Gleave

Dr Frank Mechan, Jack Gillespie, Giorgio Praulins

BILL & MELINDA
GATES *foundation*



Background

- Despite clear guidelines there is a high level of noise in bioassays.
- **Noise – variation caused by factors other than the test item.**
- Defining precision as the consistency and reproducibility of bioassays.

Aims

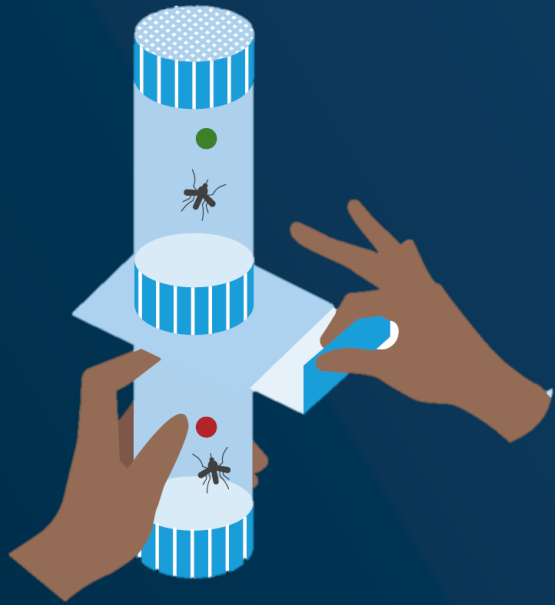
- Quantify the precision of standard mosquito bioassays
- Identify the sources variation in bioassays
- Produce guidance on reducing the level of noise



Method

WHO Tube Bioassay

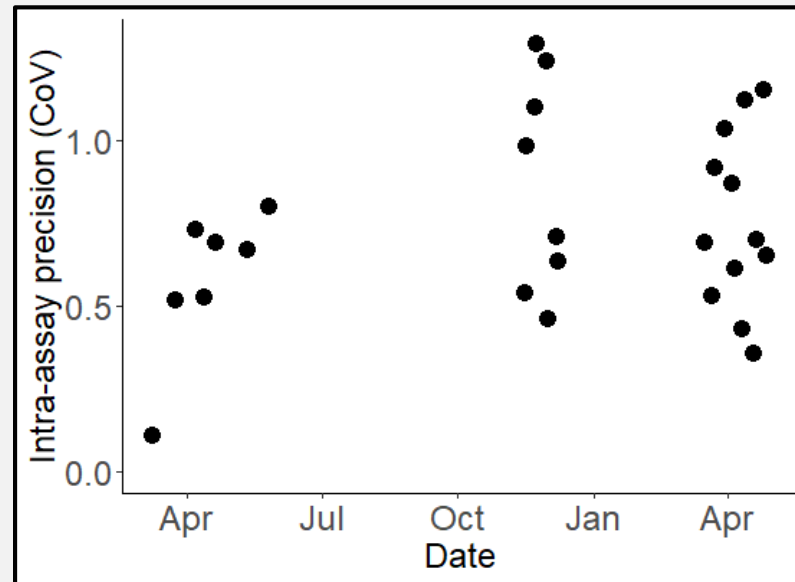
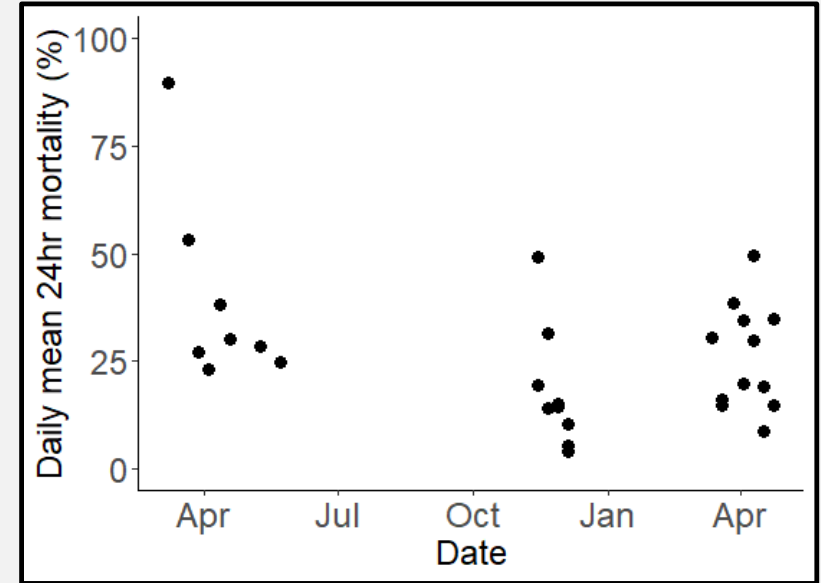
- Susceptible *An. gambiae* ('Kisumu') reared by LITE (Liverpool Insect Testing Establishment)
 - 2-5 day old, non bloodfed females
- Exposed to 0.03% permethrin (LC 50)
 - 60-minute exposures
 - 20-30 mosquitoes per tube
- 404 treated tubes total
 - **Per day:** Seven treated and two negatives
 - Two operators perform tests under same conditions



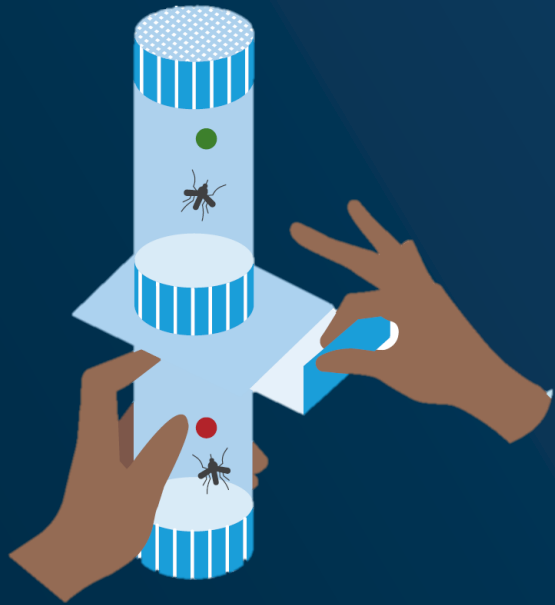
Results: overview of outcomes

GLMM – estimate the impact of each variable, with random effects for each individual assay and each testing day.

Long run mean mortality:
36.07%
(95% CI: 20.85-62.42)



Long run precision
(Coefficient of Variation):
0.76

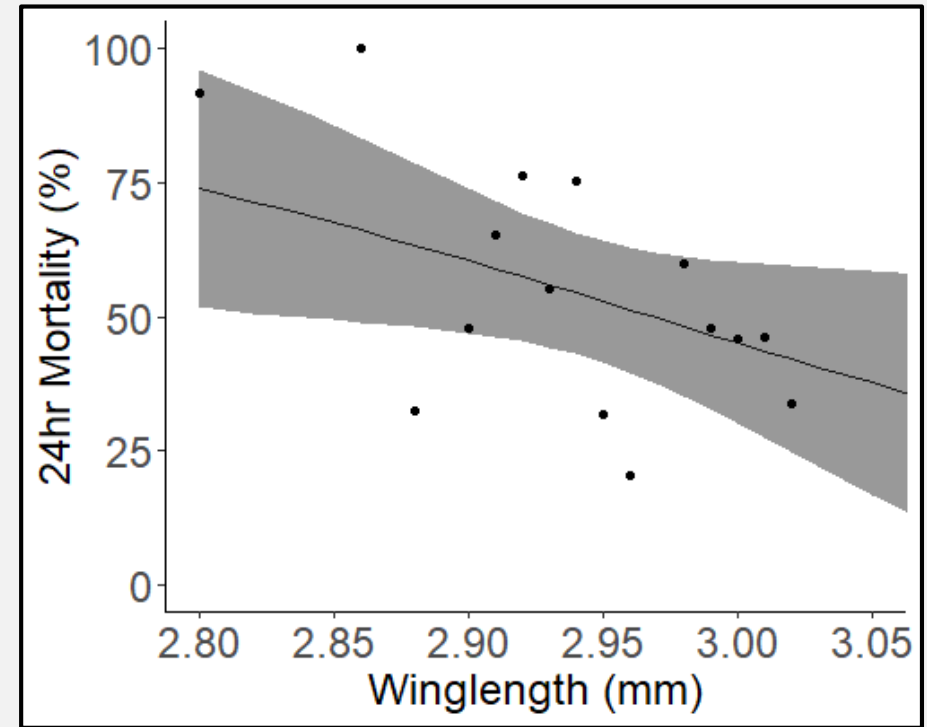


Results: sources of variation

Winglength

Negative effect on mortality
($p < 0.001$).

Increase from 2.94mm (mean) to
3.01mm (1 SD higher) results in
10.72% decrease in mortality.



Total number

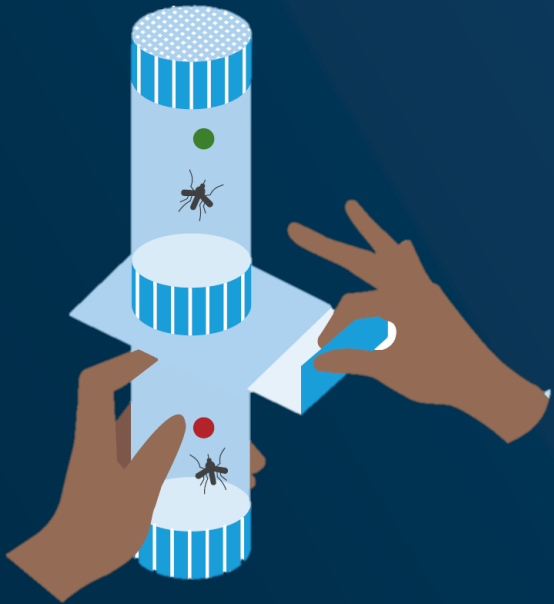
Significant positive effect on mortality ($p = 0.044$)

Each additional mosquito above 25 increased mortality by 1.90%.

Environmental conditions

Relative humidity (either the start or end of exposure)
did not significantly impact mortality ($p = 0.07$)*

*Meaning no effect within the ranges of values observed



Why is understanding variation important?

The variability of an assay impacts how difficult it is to identify the underlying 'truth'.

More variability means that more samples needed to detect smaller differences.

But how many?

And what is the smallest difference we can detect when increasing sample size is not feasible?



Improved guidance for assessing PBO synergism

- Currently tube assay used to assess if wild populations show signs of metabolic resistance.
 - Pyrethroid vs pyrethroid + synergist
- However, **sample size predetermined** by guidance
 - 4x4 – four tubes of each treatment
 - Can this detect smaller differences?
- Need ‘rules of thumb’ for assessing credibility of results
 - *‘only a mortality difference of >X% can be **reliably detected**’*

So.....

We need better understanding of power in WHO tubes assays

But....

- Difficult to communicate interaction of many variables
- More complex power analysis risks being less accessible.

Aims:

Identify minimum mortality improvement detected by '4x4'.

- Quantify the impact of within-day and between-day variance.
- Quantify the impact of increasing tube number.

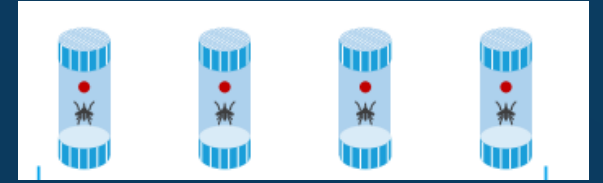
...And in the process make methods for performing power analysis easy and accessible

Methods:

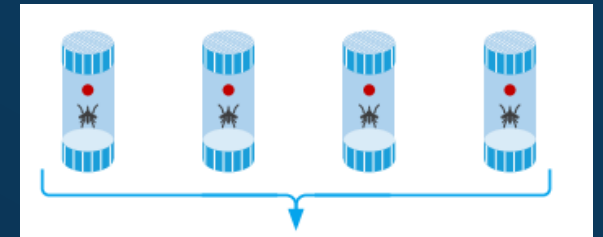
Identifying a minimum mortality improvement ('threshold') for PBO synergism requires large-scale power simulations.

- Probability of detecting given effect sizes quantified for many different hypothetical experimental designs.
 - Different number of tubes
 - across a range of variance values for both:
 - Tubes on same day
 - Tubes on different days

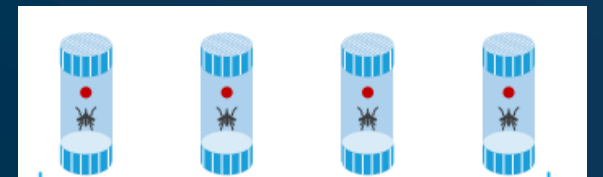
Pyrethroid



PBO



Pyrethroid



(based on the variability we observed)

Key findings

If all assays performed on same day

A '4x4' design can reliably detect a 25% difference

A '5x5' design can reliably detect a 20% difference

Detecting a 15% difference requires a '9x9' design

...However, if assays are spread over multiple days, it becomes harder to detect the same effect size

Very broadly, spreading the assays over multiple days means difference must be 5% larger to be detected

Key Discussion point

Current guidance suggests $\geq 10\%$ difference indicates synergism. However, a 4x4 design is not powered to detect this.





Thank you