New WHO procedures and insecticide discriminating concentrations for monitoring resistance in adult mosquito vectors

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&

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World Health Organization

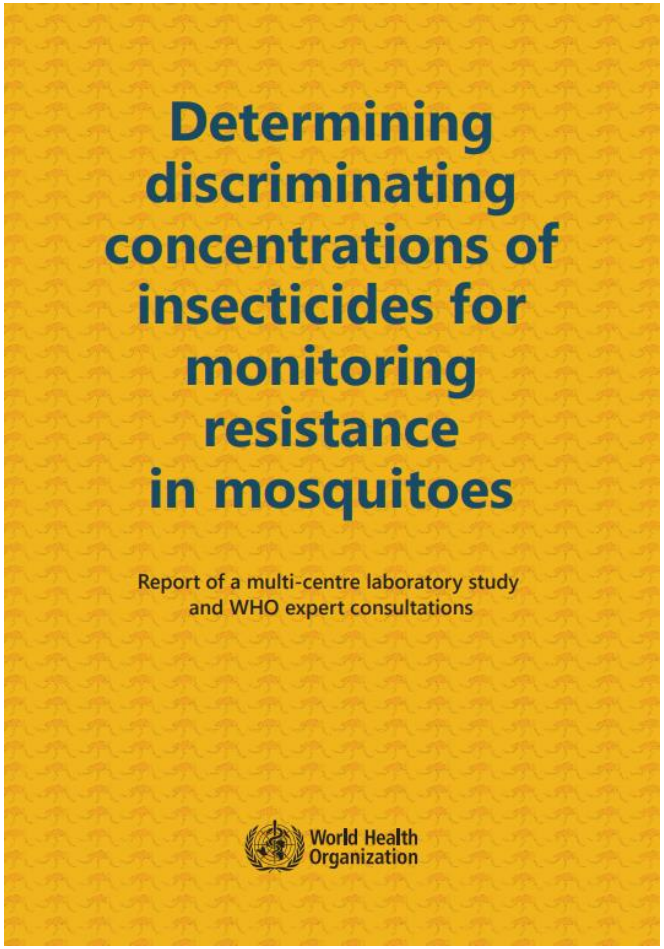
WHO multi-center study to establish new DCs and procedures

Study duration

- 2017 – 2021
- 23 participating laboratories in five WHO regions

Key outcomes

- Built capacity of laboratories on test methods & procedures
- Established and validated 17 new insecticide DCs for *Aedes* spp.
- Established and validated 13 new DCs for *Anopheles* spp.
- Developed and validated the new standard bottle bioassay method, “the WHO bottle bioassay”,
- Developed the first version of the SOPs
- Created a database of bioassay records of concentration–response tests for > 400 000 mosquitoes including source of variations in test outcomes under different test conditions
- Identified and recommended measures to improve laboratory testing procedures and to guide the selection of DCs of insecticides commonly used for vector control.
- Identified knowledge gaps for future work.

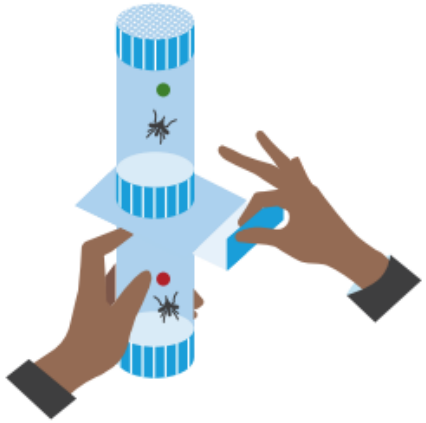


https://apps.who.int/iris/handle/10665/352616

New DCs for WHO tube test

Anopheles

Insecticide	Species	Discriminating concentration	Carrier oil or solvent
Alpha-cypermethrin	*An. albimanus* and *An. stephensi*	0.30%	Silicone oil
	An. funestus, An. minimus and *An. gambiae*	0.05%	
Pirimiphos-methyl[a]	*An. albimanus, An. stephensi, An. minimus* and *An. funestus*	100 mg/m^2	Acetone alone
	An. gambiae	170 mg/m^2	



Aedes

Insecticides	Species	Discriminating concentration	Carrier oil or solvent
Alpha-cypermethrin	*Ae. aegypti*	0.05%	Silicone oil
	Ae. albopictus	0.08%	
Bendiocarb	*Ae. aegypti* and *Ae. albopictus*	0.20%	Olive oil
Chlorpyrifos-ethyl	*Ae. aegypti* and *Ae. albopictus*	1%	Olive oil
Permethrin (40:60[a])	*Ae. aegypti* and *Ae. albopictus*	0.40%	Silicone oil
Deltamethrin	*Ae. aegypti* and *Ae. albopictus*	0.03%	Silicone oil
Lambda-cyhalothrin	*Ae. aegypti*	0.05%	Silicone oil
	Ae. albopictus	0.08%	
Malathion	*Ae. aegypti*	1.50%	Olive oil
	Ae. albopictus	5%	
Pirimiphos-methyl[b]	*Ae. aegypti* and *Ae. albopictus*	60 mg/m^2	Acetone alone

- Species specific discriminating concentrations (DCs)
- New DCs for *Aedes* replace all previous tentative DCs
- For pirimiphos-methyl, programs and partners are advised to validate results obtained with the former tentative DC against the new DC

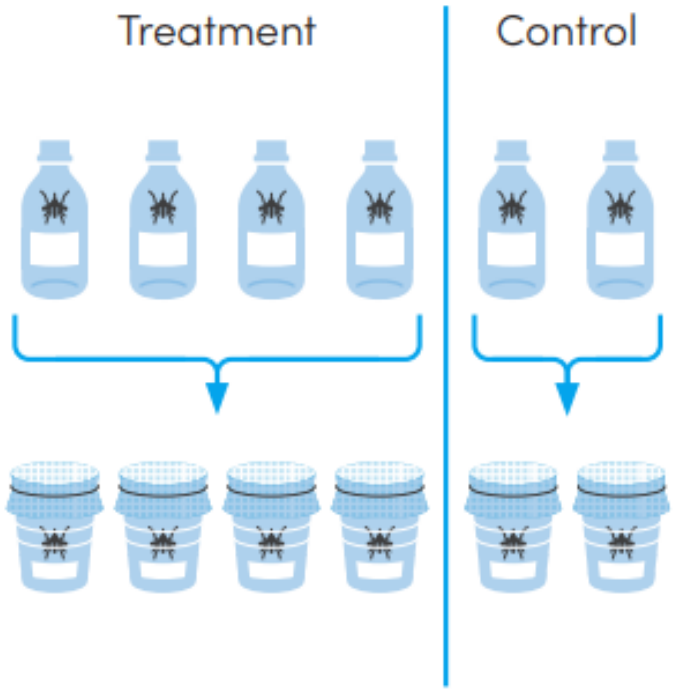
The new WHO bottle bioassay

- **Developed for insecticides that cannot be impregnated on filter papers:** transfluthrin, prallethrin, metofluthrin, clothianidin, flupyradifurone, chlorfenapyr, pyriproxyfen

- **Similar to the CDC bottle bioassay but with endpoints aligned with those of WHO tube tests:**

Exposure period (1 h):
Introduce 25 mosquitoes into each treatment and

Holding period (24 h or 72 h):
Transfer mosquitoes into the holding paper cups. Provide them access to a water-sugar solution. Read mortality at the end of the 24 h (or 72 h for chlorfenapyr)



DCs based on the WHO bottle bioassay

Aedes

Insecticide	Species	Discriminating concentration (µg/bottle)	Solvent and surfactant
Clothianidin	*Ae. aegypti*	20	Acetone + MERO 1500 ppm
	Ae. albopictus	10	
Flupyradifurone	*Ae. aegypti* and *Ae. albopictus*	80	Acetone + MERO 1500 ppm
Metofluthrin	*Ae. aegypti* and *Ae. albopictus*	1	Acetone
Prallethrin	*Ae. aegypti* and *Ae. albopictus*	30	Acetone
Transfluthrin	*Ae. aegypti* and *Ae. albopictus*	3	Acetone

MERO (surfactant) was used to allow for adequate coating of bottles and prevent crystallization of technical materials (insecticide active ingredients)

Anopheles

Insecticide	Species	Discriminating concentration (µg/bottle)	Bottle drying time (h)	Recording/ holding time (h)	Solvent/ surfactant
Clothianidin	*An. albimanus*	10	24	24	Acetone + MERO 200 ppm
	An. stephensi	10	24	24	Acetone + MERO 800 ppm
	An. funestus and *An. gambiae*	4	24	24	
	An. minimus	6	24	24	
Flupyradifurone	*An. albimanus*	500	24	24	Acetone + MERO 200 ppm
	An. stephensi and *An. gambiae*	60	24	24	Acetone + MERO 800 ppm
	An. funestus and *An. minimus*	100	24	24	
Transfluthrin	*An. albimanus*, *An. stephensi*, *An. funestus*, *An. minimus* and *An. gambiae*	2	24	24	Acetone
Chlorfenapyr	*An. gambiae*, *An. stephensi*, *An. funestus* and *An. albimanus*	100	24	72	Acetone
Pyriproxyfen[a]	*An. gambiae*, *An. stephensi* and *An. funestus*	100	2	7 d[b]	Acetone

Automated spread sheets for preparing stock solutions

Available at: https://cdn.who.int/media/docs/default-source/ntds/vector-ecology-mangement/calculation-tables-paper-impregnation-bottles-17jan2022-locked.xlsx?sfvrsn=83a556a4_7

Calculation for weight of insecticide AI for coating glass bottles with or without a surfactant

Enter name or value of a variable in light green cells; white cells are locked and automatically calculate values

Insecticide class group	Surfactant	Insecticide	Targeted concentration of AI in the bottles (µg /bottle)	No. of bottles to be coated	Amount of surfactant per bottle* (ppm or µg)	Total weight of surfactant needed (mg)	Density of surfactant	Volume of surfactant (mL)	Volume of acetone (mL)	Total volume of coating solution (mL)	Calculation for AI weight in g adjust: Amount of AI to weigh for coating bottles (g)	Purity of insecticide AI (%)	Adjusted amount of AI to weigh (g)*
			a	b	c	d = (b × c)/1000	e	f = (d/e) / 1000	g = (b × 1) - f	h = f + g	i = (a x b) / 10^6	j	k = i x (100 / j)
	e.g. MERO[a]			1	800	0.8	0.900	0.001	1.00	1	0.000000	99.2	0.000000
		Control		1	800	0.8	0.900	0.001	1.00	1			
	None[b]			1					1.00	1	0.000000	99.8	0.000000
		Control		1					1.00	1			

AI, active ingredient

MERO, 81% rapeseed oil methyl ester

*Considering purity of the insecticide AI

**Exact weight of AI shown on the electronic balance

***This is the volume adjusted for exact weight of AI; use 1 mL solution to coat a 250 mL bottle

Operating Manual | A1-Calculation with oil | A2-Calculation without oil | A3- Stock solution paper imprg. | B1- Calculation for bottles | B2- Stock solution bottles

WHO bottle bioassay with Chlorfenapyr

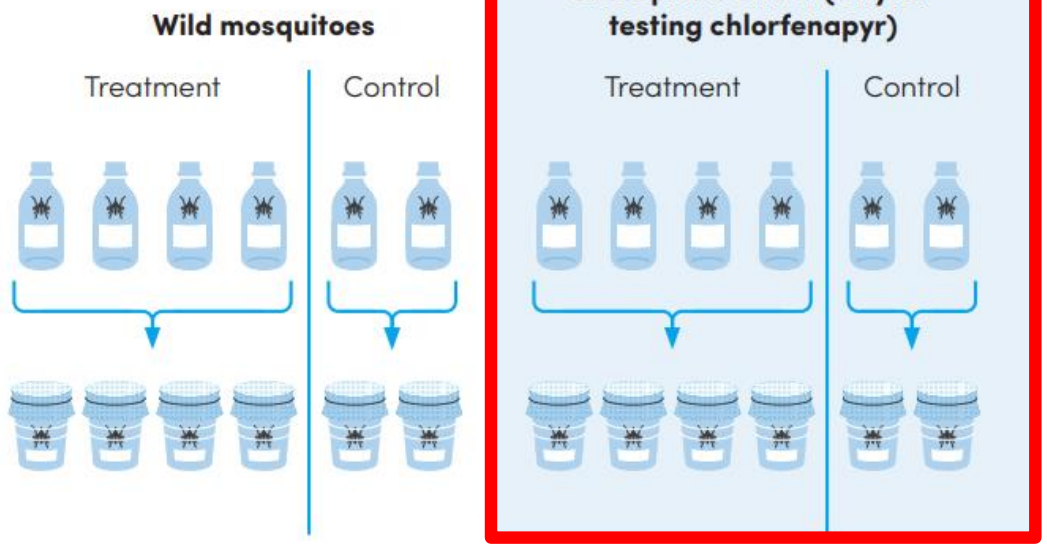
PROCEDURE

- Test to be conducted strictly at: 27 °C ± 2 °C
- Susceptible colony mosquitoes to be tested in parallel

= 25x

Exposure period (1 h): Introduce 25 mosquitoes into each treatment and

Holding period (24 h or 72 h): Transfer mosquitoes into the holding paper cups. Provide them access to a water-sugar solution. Read mortality at the end of the 24 h (or 72 h for chlorfenapyr)



CONFIRMING RESISTANCE

Three bioassays with same mosquito population for the same site and at different time points are needed to confirm resistance. Resistance is confirmed when:

• the mortality of test mosquitoes 72 h post-exposure is <90% in all 3 tests

• the mortality in the susceptible laboratory colony, tested in parallel to the wild mosquitoes, is ≥98% in all 3 tests

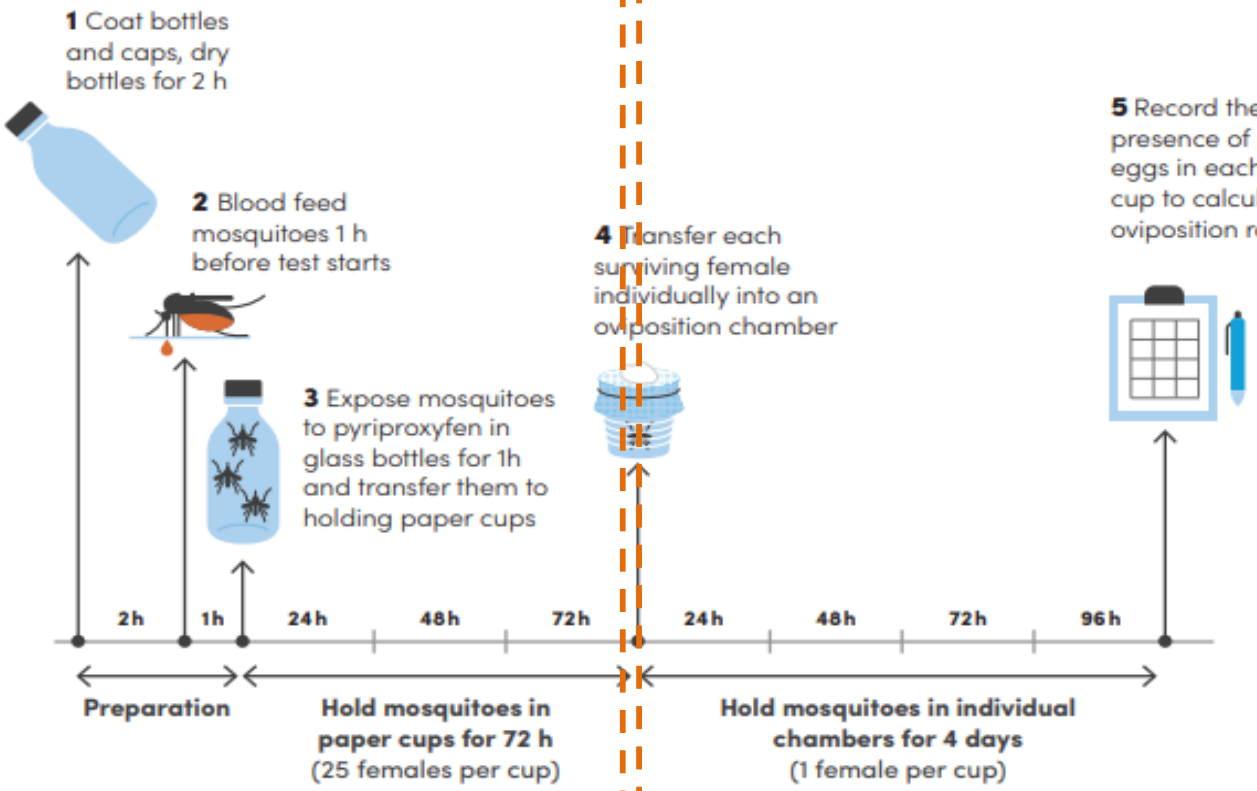
WHO bottle bioassay with Pyriproxyfen

First part of the test is a WHO bottle bioassay but with:

- Blood fed mosquitoes
- Mortality measured at 24h, 48h and 72h

Second part of the test is new:

- Chambering mosquitoes individually
- Counting the numbers that lay eggs

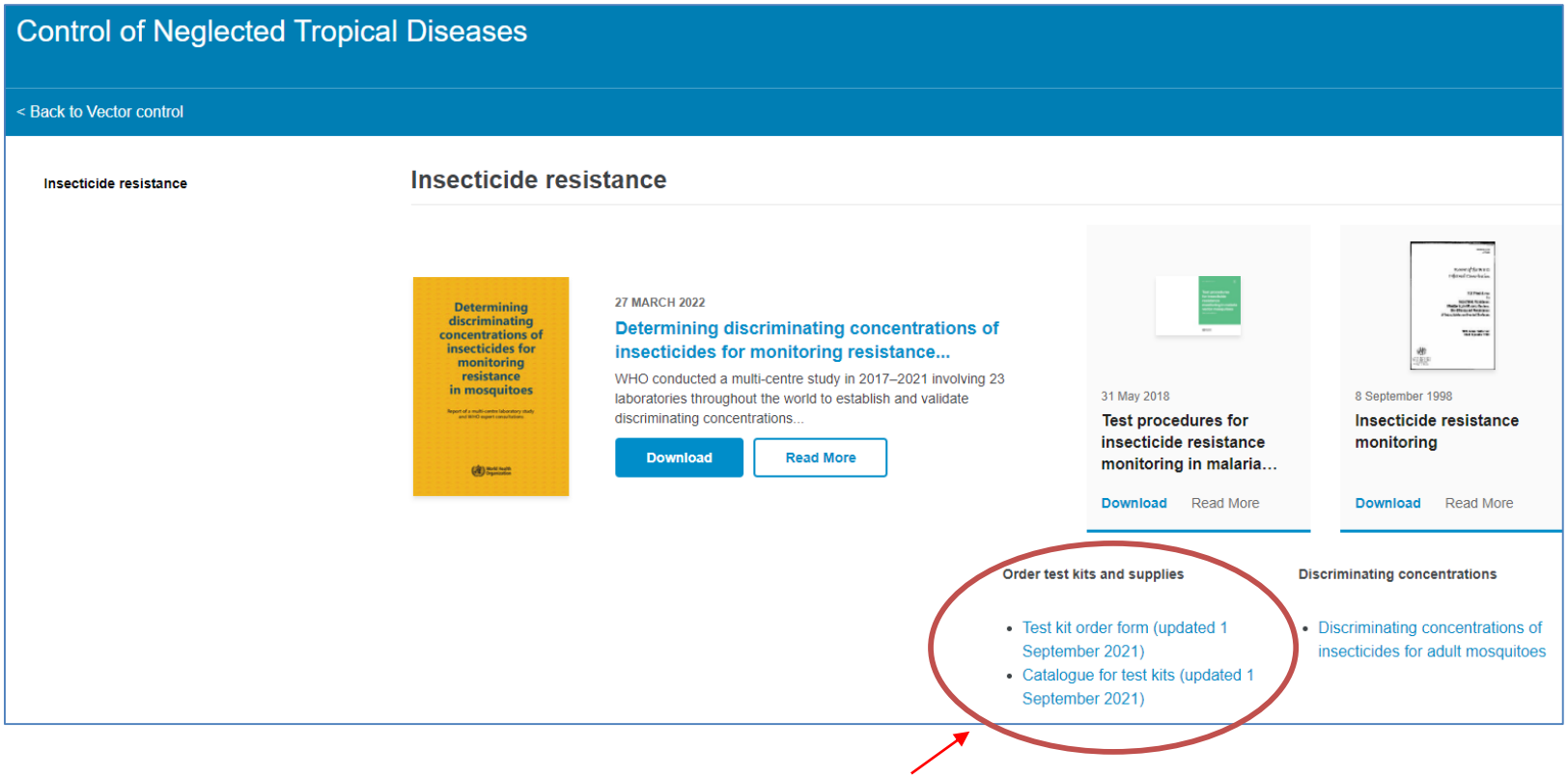


End point: oviposition inhibition

The process is done with mosquitoes from field and a susceptible colony in parallel

Ordering test kits and impregnated papers

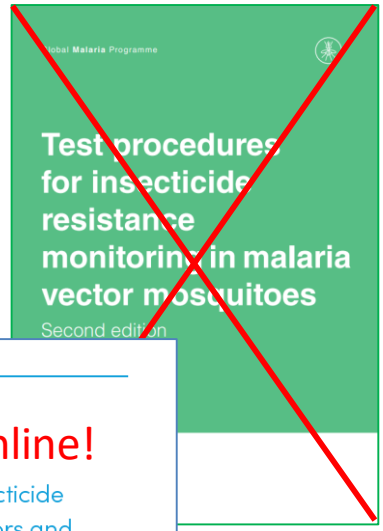
The catalogue of the Universiti Sains Malaysia (USM) is being updated



Form and catalogue available at: https://www.who.int/teams/control-of-neglected-tropical-diseases/vector-ecology-and-management/vector-control/insecticide-resistance

New insecticide resistance monitoring manual

- **Replaces** the former 'Test procedures for insecticide resistance monitoring in malaria vector mosquitoes'
- **Integrates** *Anopheles*, *Aedes* and *Culex* spp.
- **Includes:**
  - 27 DCs for *Anopheles* (13 of them new) and 13 DCs for *Aedes*
  - Description of the WHO bottle bioassay and special requirements and processes for chlorfenapyr, pyriproxyfen
  - Prioritizing resistance tests amid limited resources and mosquitoes
- **Provides new guidance** on:
  - Testing a representative sample of mosquitoes
  - Data management and reporting
  - Using resistance data for decision making



New Standard Operating Procedures (SOPs)

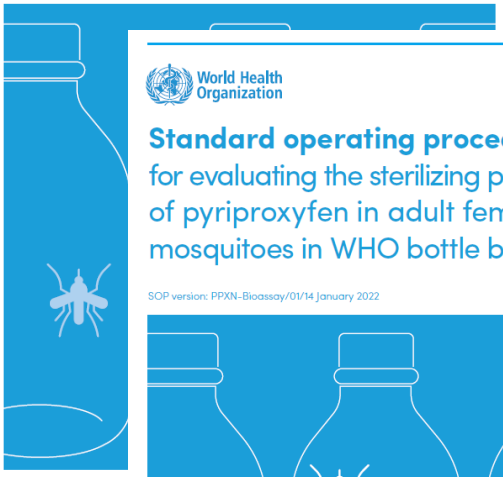
Provide step by step instruction to conduct all WHO susceptibility bioassay. A tool for field technicians that are running the bioassays.

Available at:
WHO IRIS or
https://www.who.int/teams/global-malaria-programme/prevention/vector-control/insecticide-resistance

World Health Organization

Standard operating procedure
for testing insecticide susceptibility of adult mosquitoes in WHO bottle bioassays

Version: WHO Bottle-bioassay/01/14 January 2022



World Health Organization

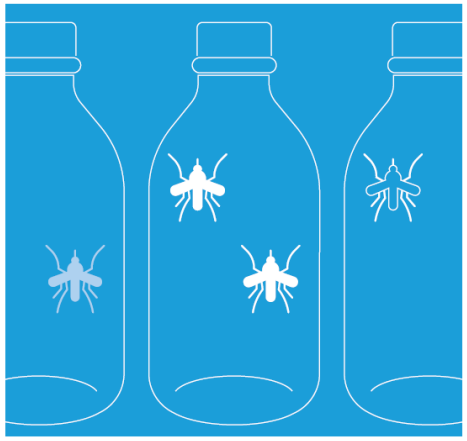
Standard operating procedure
for testing insecticide susceptibility of adult mosquitoes in WHO tube tests

SOP version: WHO Tube test/01/14 January 2022

World Health Organization

Standard operating procedure
for evaluating the sterilizing properties of pyriproxyfen in adult female mosquitoes in WHO bottle bioassays

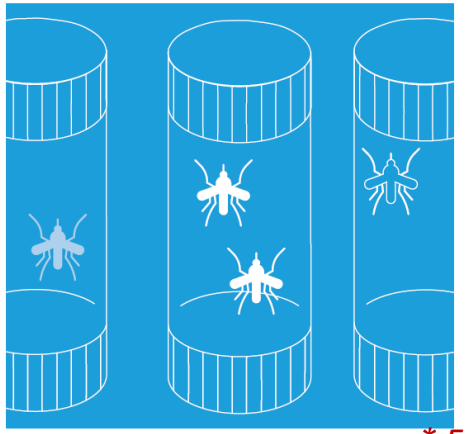
SOP version: PPXN-Bioassay/01/14 January 2022



World Health Organization

Standard operating procedure
for determining the ability of PBO to restore susceptibility of adult mosquitoes to pyrethroid insecticides in WHO tube tests

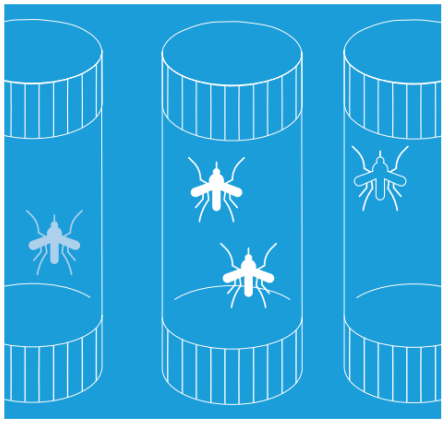
WHO SOP version: PBO-insecticide synergist bioassay/01/14 January 2022



World Health Organization

Standard operating procedure
for impregnation of filter papers for testing insecticide susceptibility of adult mosquitoes in WHO tube tests

SOP version: Paper-Impreg/01/14 January 2022



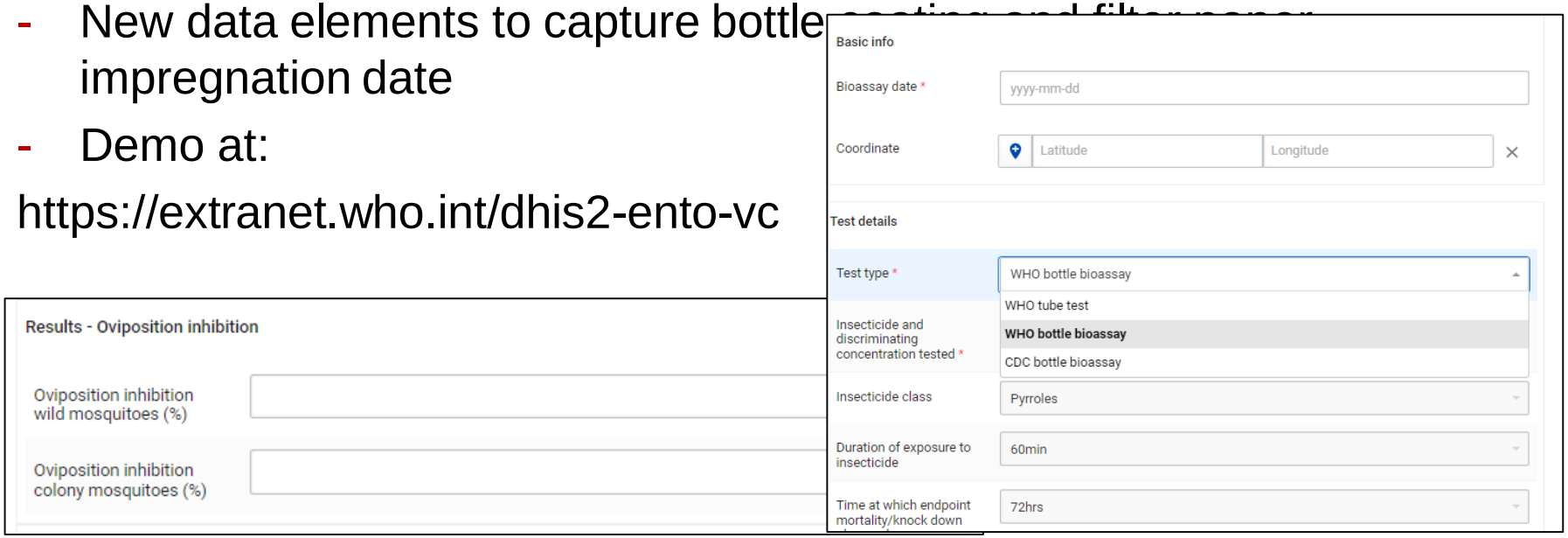
* *For research & product development purposes*

Update DHIS2 modules for resistance monitoring

Include:

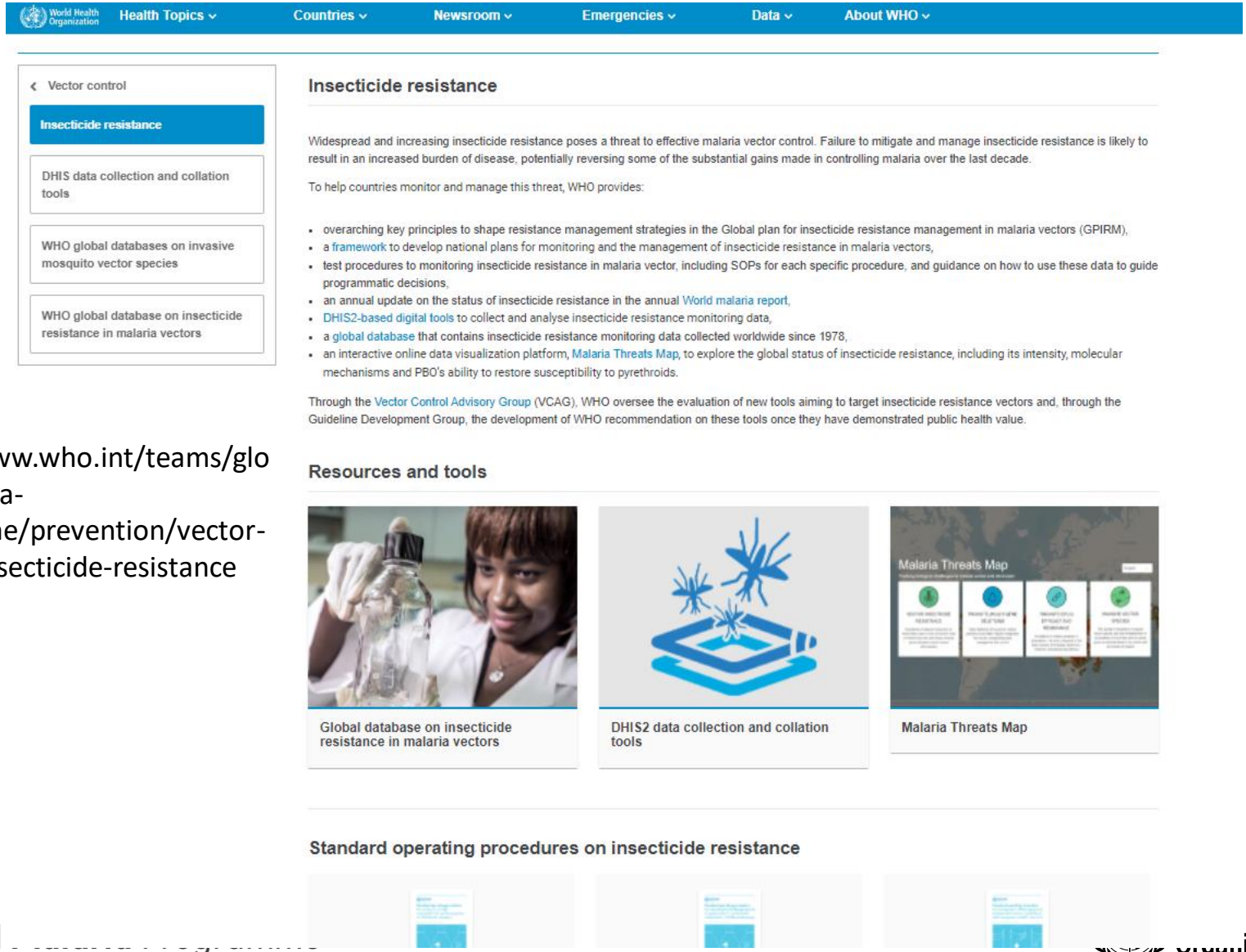
- New insecticide DCs (enhanced option set)
- New data elements for bioassay with chlorfenapyr and pyriproxyfen
- New data elements to enter data per replicate (tube or bottle bioassay)
- New data elements to record temperature during bioassay
- New data elements to capture bottle coating and filter paper impregnation date
- Demo at:

https://extranet.who.int/dhis2-ento-vc



New GMP webpage on insecticide resistance in malaria vectors

Bringing all resources together to improve accessibility:



Link:
https://www.who.int/teams/global-malaria-programme/prevention/vector-control/insecticide-resistance

Updated NTD page on insecticide resistance

Control of Neglected Tropical Diseases

< Back to Vector control

Insecticide resistance

Insecticide resistance

To be replaced by new manual soon

27 MARCH 2022

Determining discriminating concentrations of insecticides for monitoring resistance...

WHO conducted a multi-centre study in 2017–2021 involving 23 laboratories throughout the world to establish and validate discriminating concentrations...

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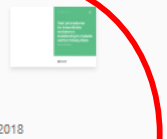

31 May 2018

Test procedures for insecticide resistance monitoring in malaria...

Download | Read More

8 September 1998

Insecticide resistance monitoring

Download | Read More

Order test kits and supplies

- Test kit order form (updated 1 September 2021)
- Catalogue for test kits (updated 1 September 2021)

Discriminating concentrations

- Discriminating concentrations of insecticides for adult mosquitoes

Standard Operating Procedures

4 MARCH 2022

Standard operating procedure for impregnation of filter papers for testing insecticide...

This SOP describes the process for impregnating filter papers with insecticides and synergists to be used in WHO tube tests for testing insecticide susceptibility...

Download | Read More

4 March 2022

Standard operating procedure for testing insecticide susceptibilit...

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4 March 2022

Standard operating procedure for testing insecticide susceptibilit...

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Acknowledgements

- **Staff from the 23 laboratories that participated in the WHO multi-centre study for establishing new DCs and SOPs:** London School of Hygiene & Tropical Medicine (Uk), Fiocruz (Brazil), Institut de Recherche en Sciences de la Santé (Burkina Faso), Organisation de coordination et de coopération pour la lutte contre les grandes endémies en Afrique Centrale (Cameroon), National Institute for Communicable Disease Control and Prevention (China), Instituto Nacional de Salud (Colombia), University of Colombia, Institut de Recherche pour le Développement(France), Malaria Research and Training Centre (Mali), (Indian Council of Medical Research–Vector Control Research Centre, Universiti Sains Malaysia, Universidad Autonoma de Nuevo Leon (Mexico), National Institute of Health (Peru), Environmental Health Institute, (Singapore), National Institute for Communicable Diseases (South Africa), Swiss Tropical and Public Health Institute, (Kasetsart University of Agriculture (Thailand), Mahidol University (Thailand), Liverpool School of Tropical Medicine (UK), Kilimanjaro Christian Medical University College (Tanzania), Centers for Disease Control and Prevention (U.S.A)
- **Experts and partners participating in WHO consultations on the results of the study**
- **Experts contributing to the development of the new WHO insecticide resistance manual and SOPs**
- **Bill & Melinda Gates Foundation for financial support**

The full list of names is provided in the multi-center study report and the new WHO manual for resistance monitoring

Thank you for your attention

Moving towards better-informed decisions