



New tools for detecting insecticide resistance: future perspective

Mark Paine on behalf of the VPMT/PQK Groups

Resistance Monitoring



Usually too late

Resistance detection methods

Test	Pros	Cons
Bioassay	Phenotype Standard methodologies	Insectary Resistance high frequency Too late for pre-emptive action Mechanism?
Biochemical	Mechanism Some prediction of cross resistance	Infrastructure Specimens stored frozen Resistance high frequency
Molecular (PCR, HOLA, SSOP)	Dried specimens Exact mechanism Mode of action (CROSS RESISTANCE) Detect at low freq (EARLY DETECTION)	Infrastructure Few specific metabolic markers

Tools for the Future

Reduce transmission of mosquito-borne pathogens

New Insecticides

Decision Support

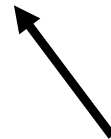
Vector
Population
Monitoring Tools

Insecticide Quantification Kits



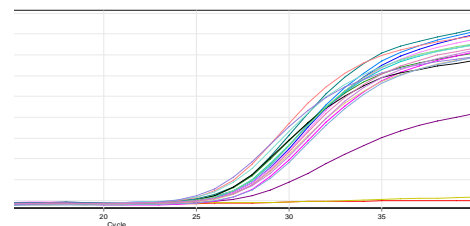
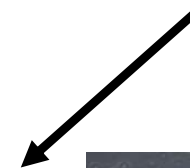
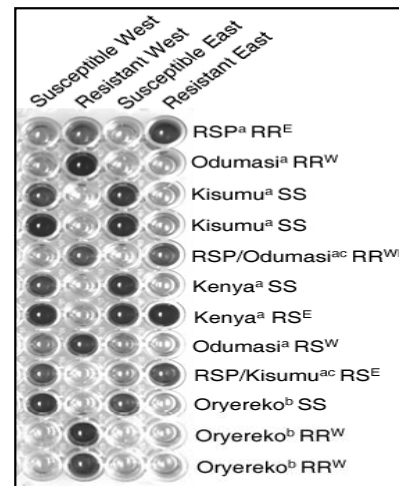
Vector Population Monitoring Tool (VPMT)

improved resistance monitoring tools that are applicable in disease endemic countries



Diagnostics

- ✓ Vector species ID
- ✓ Parasite infection
- ✓ Target-site insensitivity
- X Metabolic resistance



RR
R+
++

Current Toolkit

Anopheles gambiae

- **Knockdown-resistance**

Bass *et al Malaria Journal* 2007; Mzilahowa *et al* 2008
Med Vet Entomo; Ridl *et al Malaria Journal* 2008

- **Acetylcholinesterase**

Bass *et al* 2010 *Pesticide Biochemistry and Physiology*

- **GABA receptors**

Nikou *et al* (in prep)

- **Species identification**

Bass *et al Malaria Journal* 2008

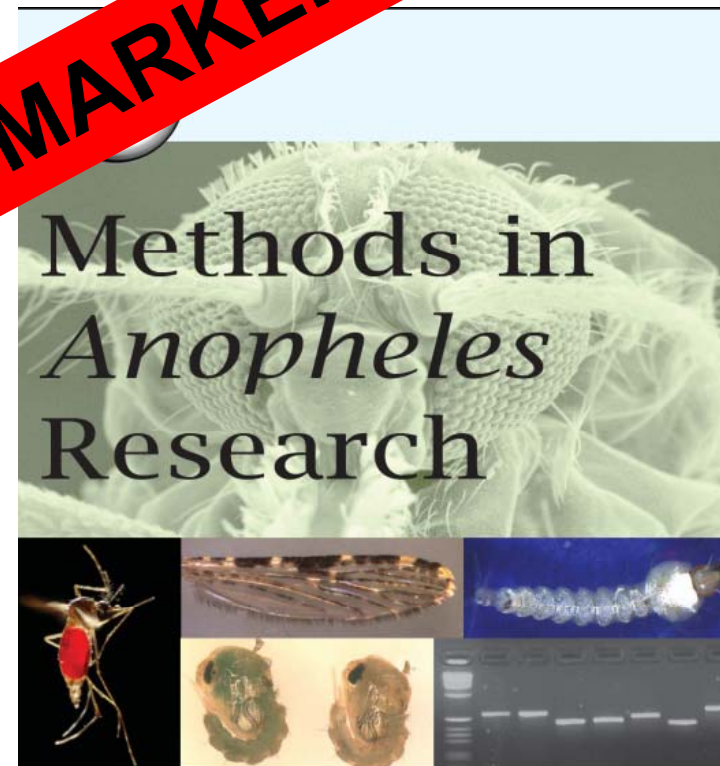
Bass *et al* *Acta Tropica* 2008

- **Plasmodium infection**

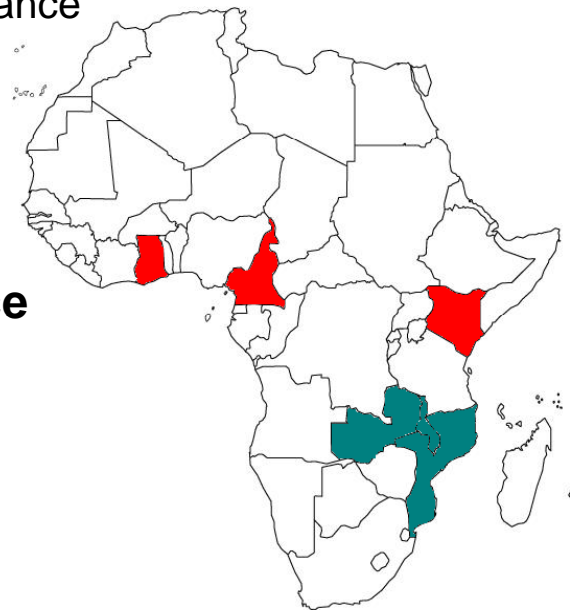
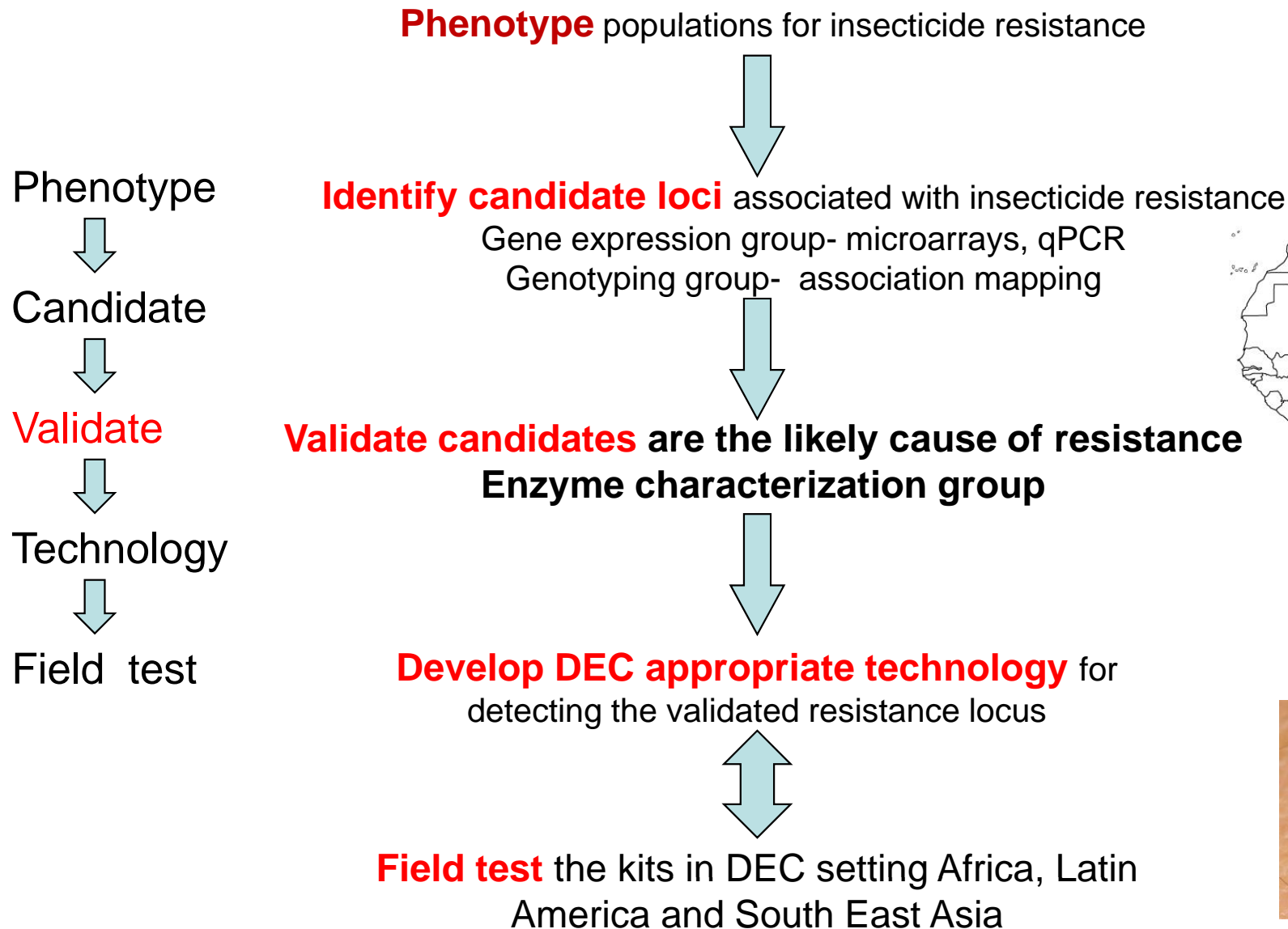
Bass *et al Malaria Journal* 2008



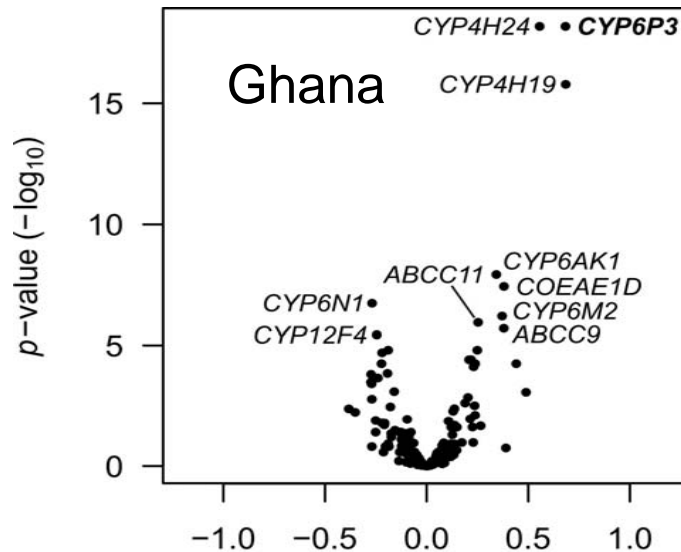
Monitoring Tool (VPMT)



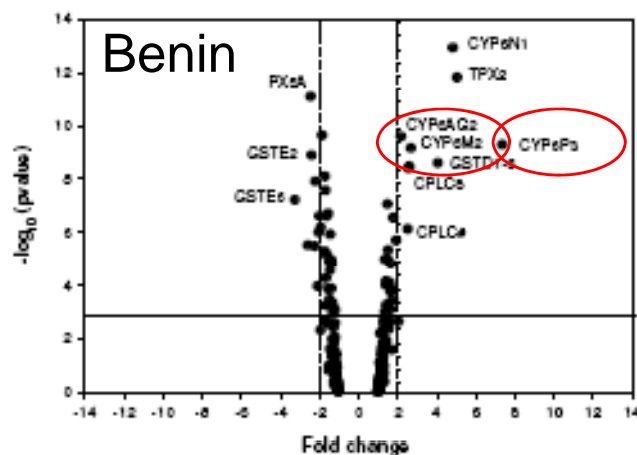
VPMT pipeline for metabolic resistance candidates



Current Status: Detox genes overexpressed in permethrin resistant *Anopheles gambiae*



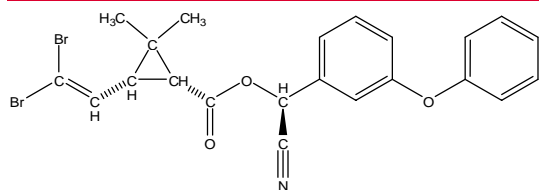
Muller et al. (2008)
PLoS Genetics



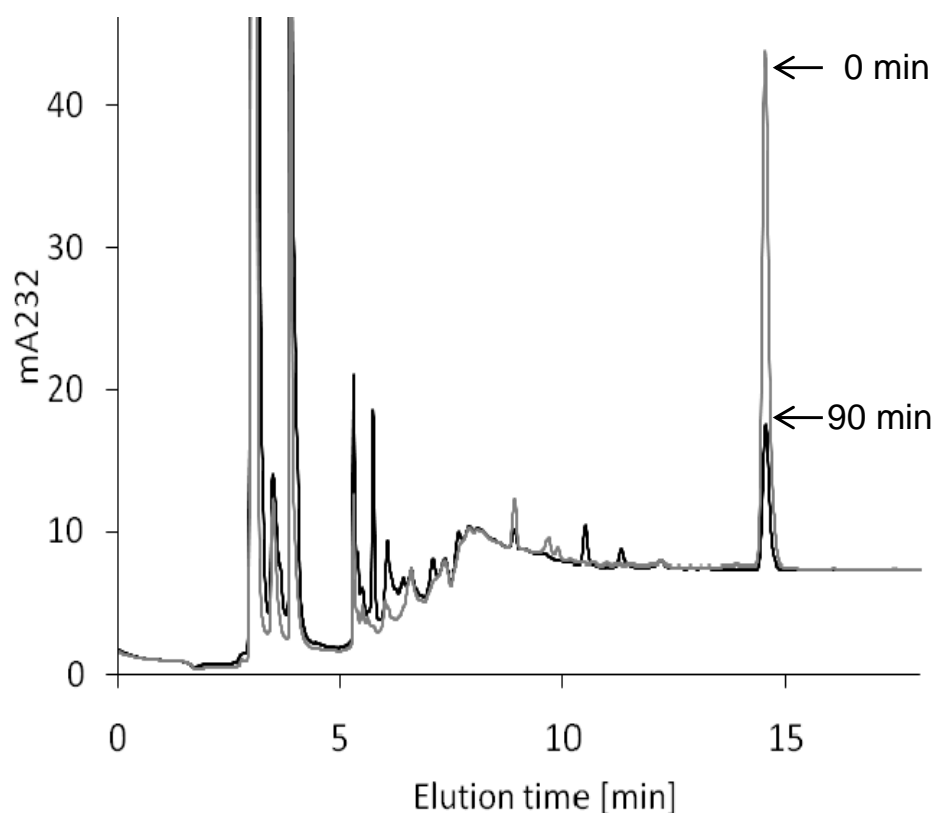
Djouaka
BMC Genomics.

P450	Location
CYP6P3	Ghana (Dodowa); Benin (Akron and Gbedjromede); Nigeria (Orogun)
CYP6M2	Ghana (Odumasy and Dodowa); Benin (Akron and Gbedjromede); Nigeria (Orogun)
CYP325A3	Kenya (Kisumu (RSP)); Nigeria (Ipokia)
CYP6Z1	Kenya (Kisumu (RSP))
CYP6Z2	Ghana (Odumasy)
CYP6AK1	Ghana (Dodowa);

Functionally validated insecticide resistance genes



Deltamethrin

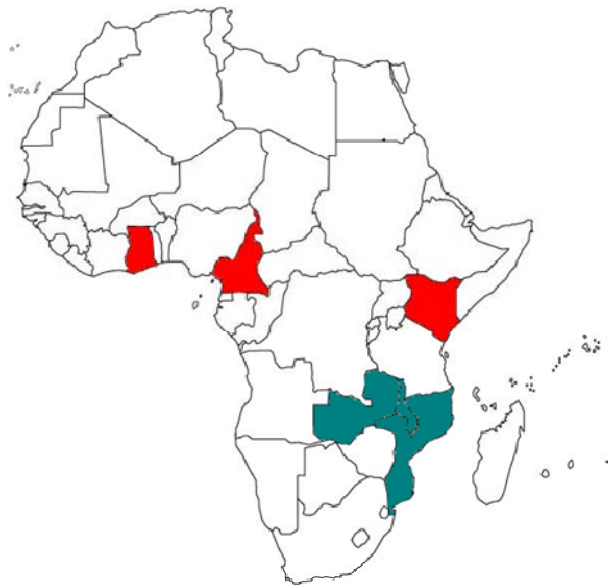


✓ Recomb. expression – Characterization:
Detailed Cross Resistance spectrum

Enzyme Activity

P450	Perm (min ⁻¹)	Delta (min ⁻¹)	DDT (min ⁻¹)
CYP6P3	3.1	2.6	0
CYP6M2	6.0	1.2	0
CYP6Z2	0	0	0

✓ Resistance genes identified



- A battery of P450s are associated with pyrethroid resistance in *An. gambiae*
- **CYP6M2 and CYP6P3** are most consistently over expressed
- ✓ They **metabolise permethrin and deltamethrin** with roughly similar kinetics

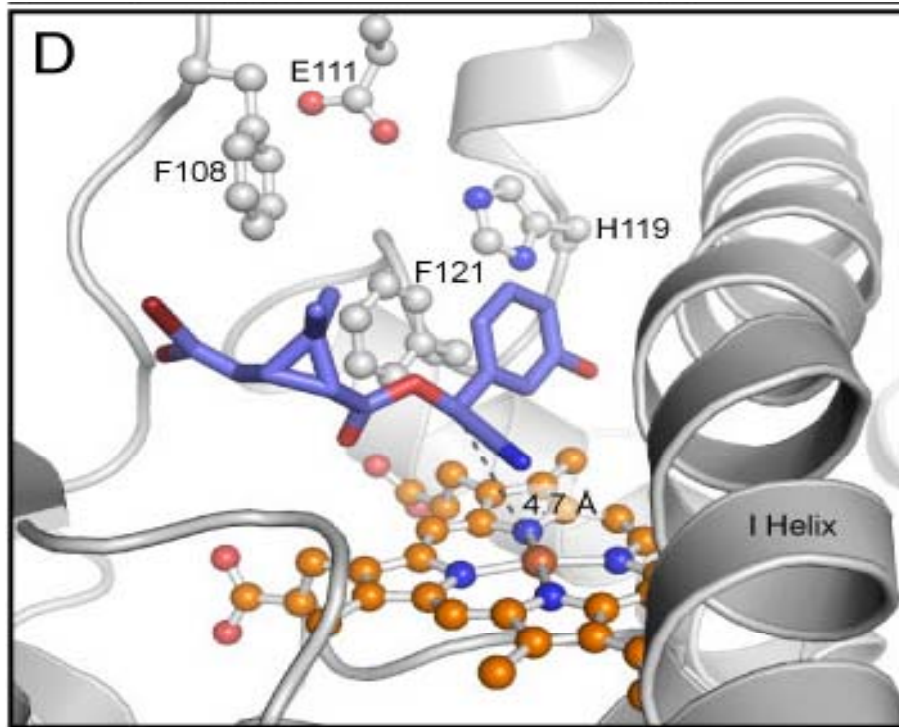
These are important markers of resistance and targets for insecticide development .

last

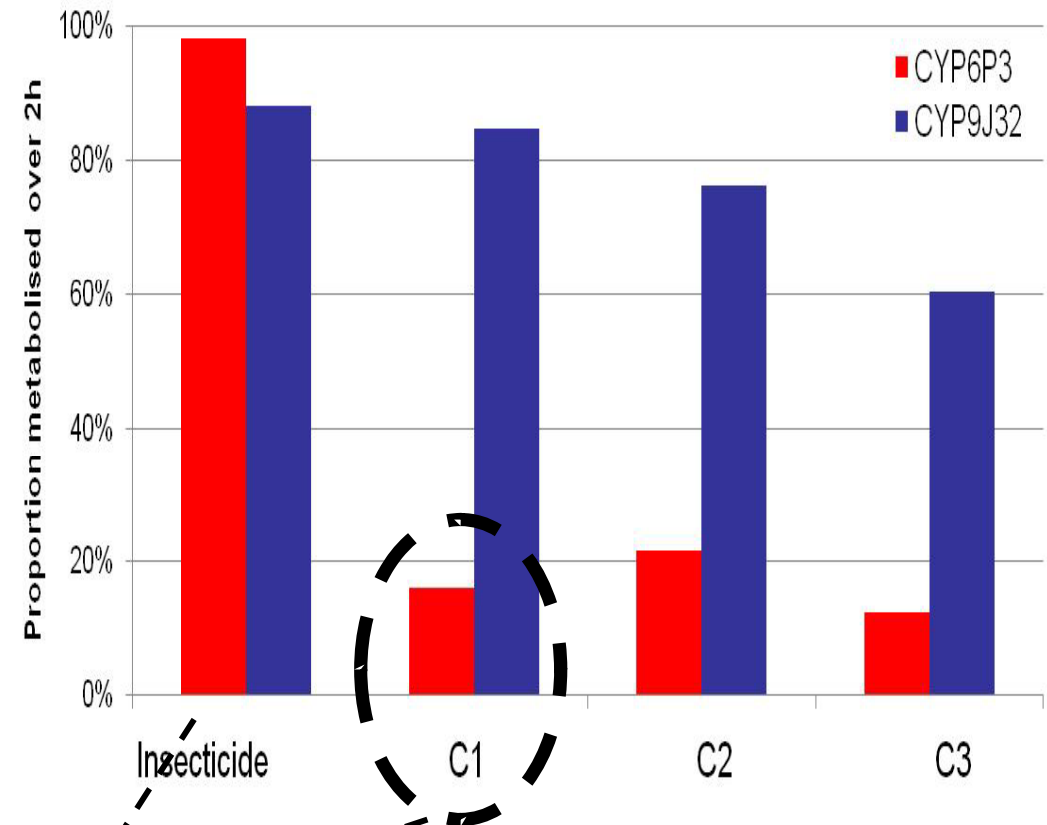


Designing insecticides that are resistant to detoxification i.e. Stick around and attack the organism

Predictive model to stymie metabolism



Experimental validation

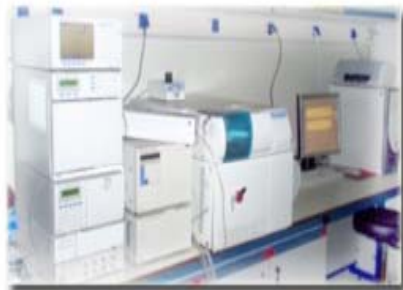


C1 is NOT metabolised by CYP6P3 – a major resistance gene
Resistance cured

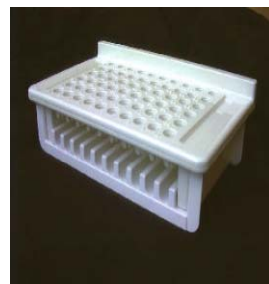
Insecticide Quality Assurance: vital element in planning successful - sustainable interventions



Available tools for measuring insecticides on ITM



HPLC-GS



Antibody (ELISA)



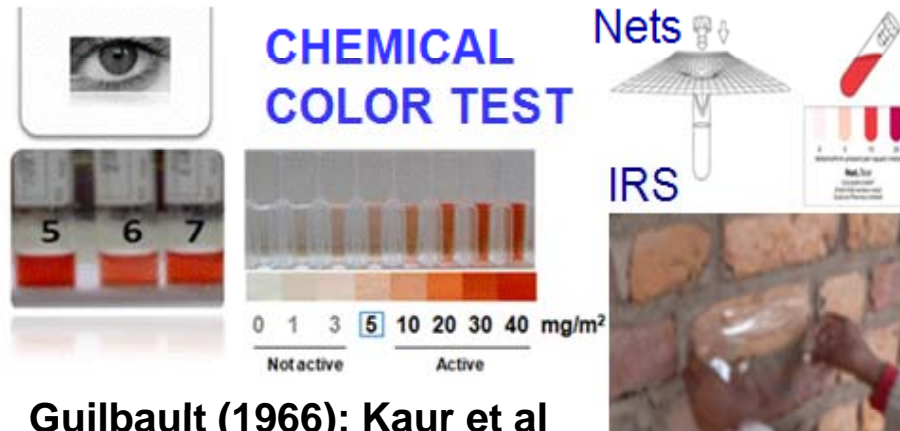
Bioassays



Expensive (>\$20) - technically demanding; skilled staff,

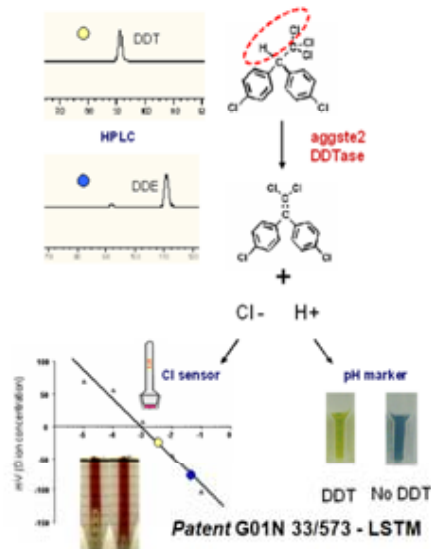
Demand for user friendly, cost-effective tests

New Technology



Guilbault (1966); Kaur et al (2009), Green et al (2009)

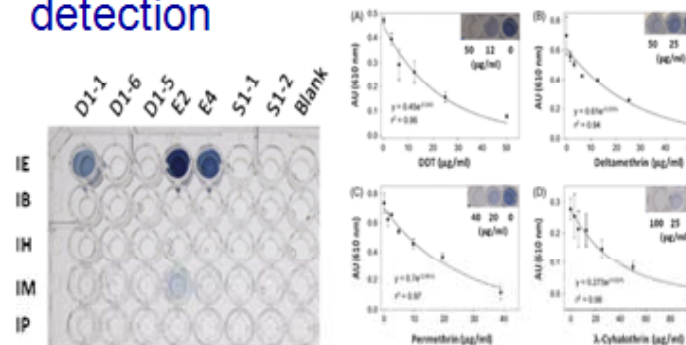
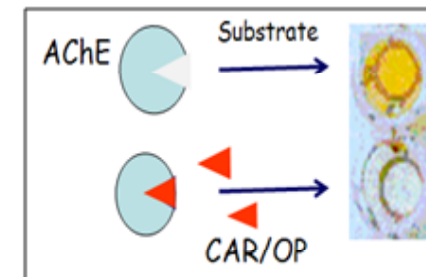
GSTe2-dehydrochlorinase biosensor assay for DDT



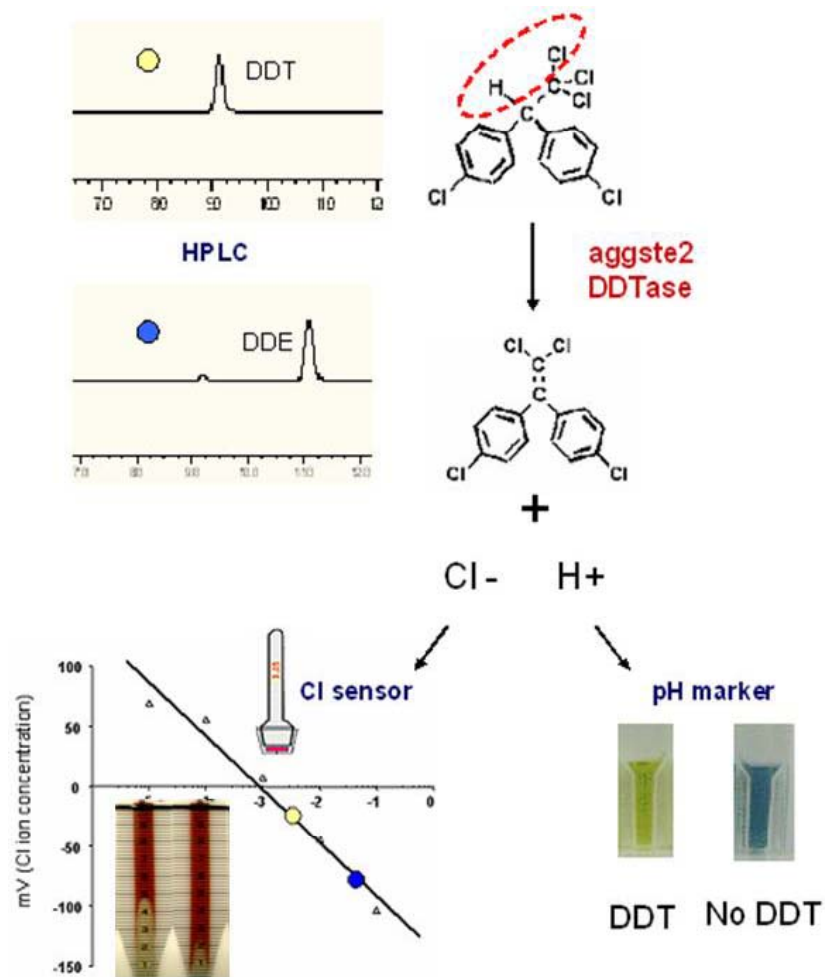
ENZYME BIOSENSORS

Enzyme (GST) based protocols for insecticide detection

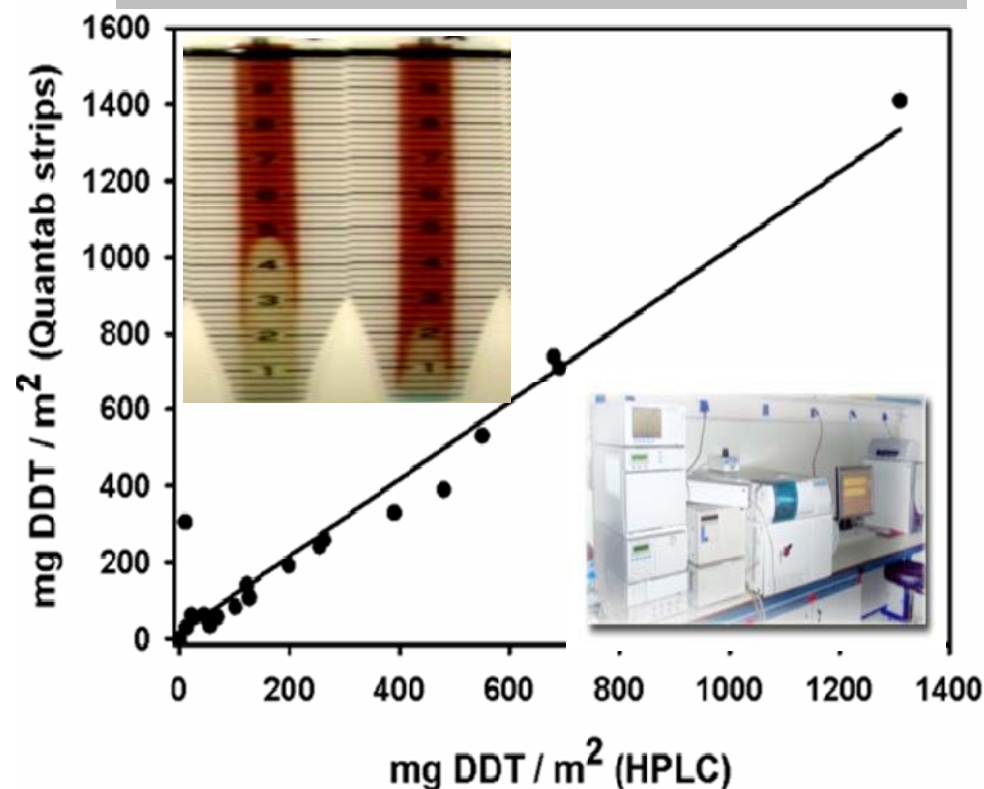
AChE: OP/bendiocarb



A GSTe2-dehydrochlorinase biosensor assay for DDT



Good correlation with HPLC in preliminary field trials (India, Uganda)



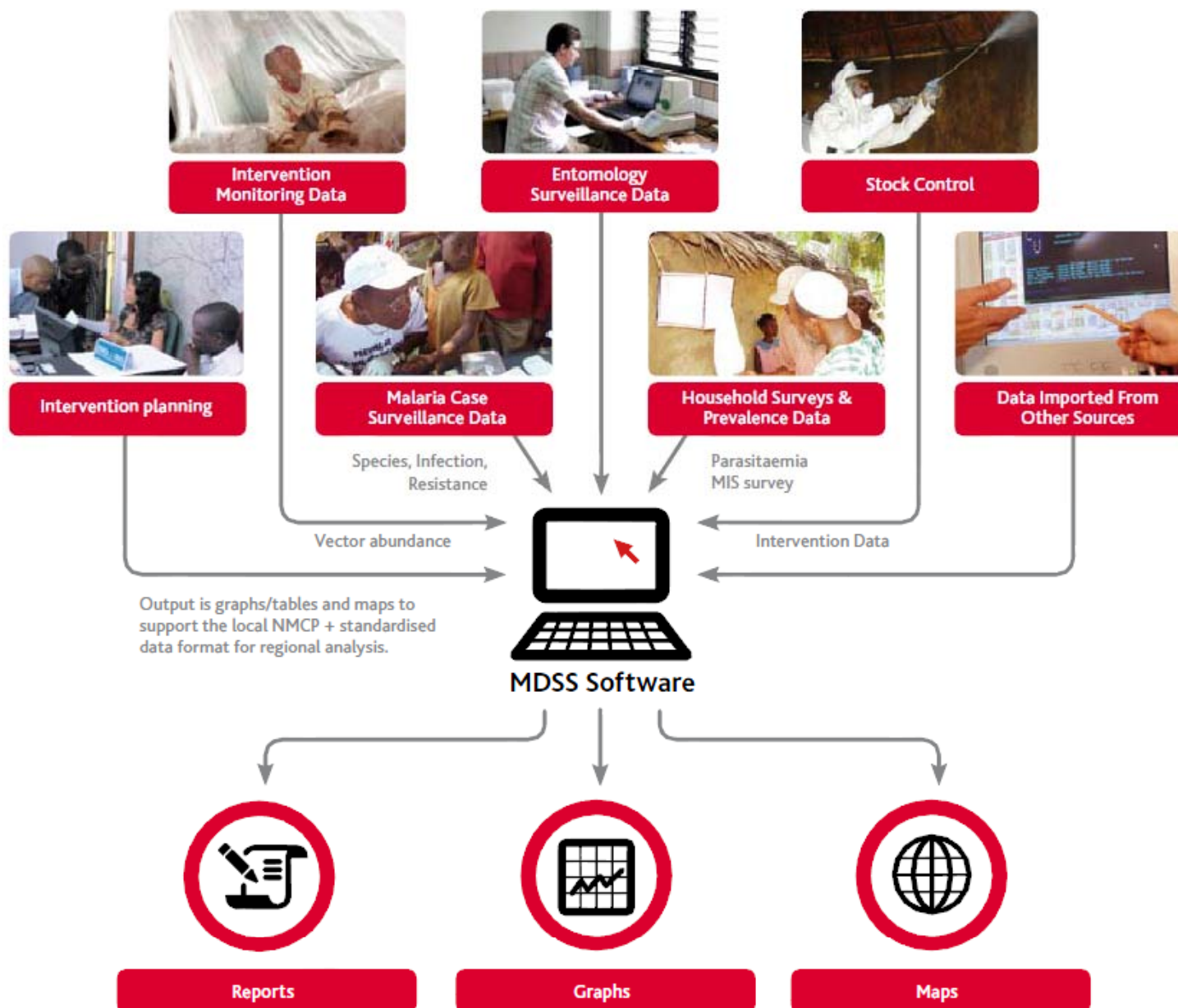
H⁺ and Cl⁻ ions released by GSTE2 are easily detected (Cl strip test or pH)

- ✓ Highly specific for the insecticidal ppDDT.

Patent G01N 33/573

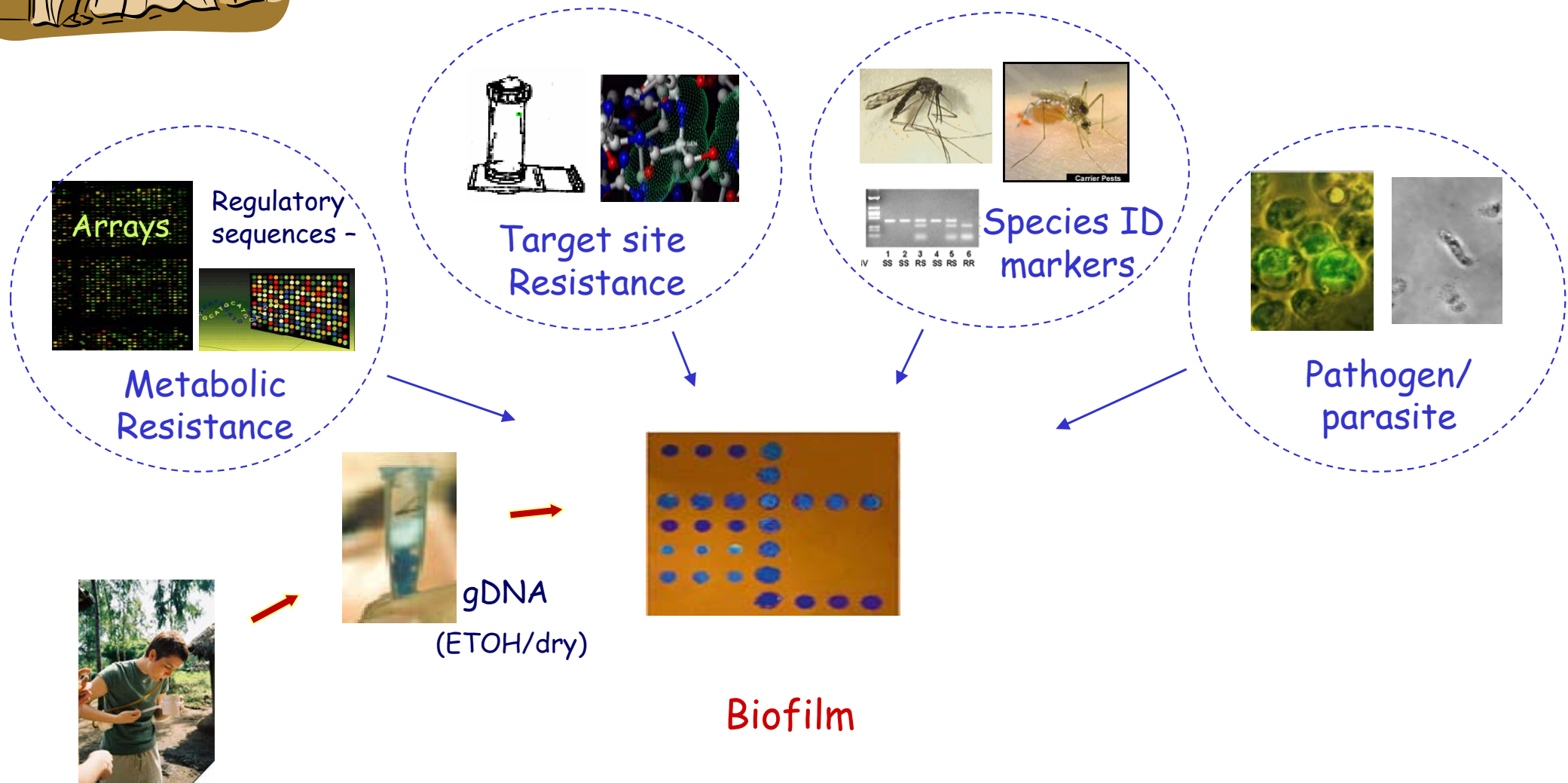
NEXT: Roll out DDT IRS Kit via WHO programs (i.e., India)

Malaria Decision Support System



Information can feed back to improve decision making in the management of malaria

Future: An integrated set of tools for speciation, infection status and resistance mechanisms



Man vs Mosquito

LSTM

Dr Martin Donnelly
Dr Hilary Ranson
Prof Janet Hemingway

SNPing/Illumina

Dr David Weetman
Dr Craig Wilding
Keith Steen

Gene expression data

Dr. Pie Miller
Dr Emma Warr

Enzyme characterization

Dr Mark Paine
Dr Bradley Stevenson
Patricia Pignatelli
Dr Andrew Dowd
Andy Steven
Hanafy Ismail

Amanda Ball
John Morgan

Rothamsted Research

Prof Lin Field
Dr Martin Williamson
Dr Chris Bass

University of Crete

Dr John Vontas
Dr Dimitra Nikou
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