Point of Care Tests for Diagnosis of Dengue Infection

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Definition - Point of Care Test

- *"An analytical test undertaken by a member of the health care team or by a non-medical individual in a setting distinct from a normal hospital laboratory."* R. Cramb – Royal Coll. Pathologists
  - Simple and rapid tests
  - Small hand-held analyzers
  - Larger desktop or portable analyzers

Diagnostic testing trends - the haves vs. the have-nots

- **Resource-rich**
  - Developed countries
  - Remote testing
  - In central hospital or commercial labs
  - Centralized, automated equipment
  - High-volume, complex test methods
  - Near-patient (POC) testing with simple or sophisticated devices
- **Resource-limited**
  - Developing countries
  - Near-patient (POC), rapid testing
  - In health clinics with no or limited lab
  - Decentralized, manual, few supplies and simple equipment
  - Low-volume, simple tests
  - Replaces syndromic diagnosis

Adapted from H. Lee, 2006

POC Test Applications

Purpose	Condition
Urgent treatment	Life-threatening illness
Starting treatment	At-risk patients
Containment	Undefined outbreaks
Failure to return	Patients lost to follow-up

Why POC testing?
"The diagnostic paradox"

- Example 1: **RT-PCR** (~1 – 2 weeks)
  - Obtain sample → ship to lab → refrigerate → batch + run test → get result → send result back to physician → notify patient → patient returns to clinic → treat. Test sensitivity 90% x Patient return 70% = **63% treated**
- Example 2: **Rapid POC test** (~1 hour)
  - Obtain sample → test on-site → get result in <1 hr → treat. Test sensitivity 70% x Patient return 100% = **70% treated**

Adapted from Gift et al 1999.

“ASSURED” – Characteristics of ideal Dx tests for the developing world

- **A**ffordable by those at risk of infection
- **S**ensitive (few false negatives)
- **S**pecific (few false positives)
- **U**ser-friendly (simple to perform)
- **R**apid treatment/**R**obust
- **E**quipment-free (no large instruments)
- **D**elivered, available to those who need it

D. Mabey et al., 2004

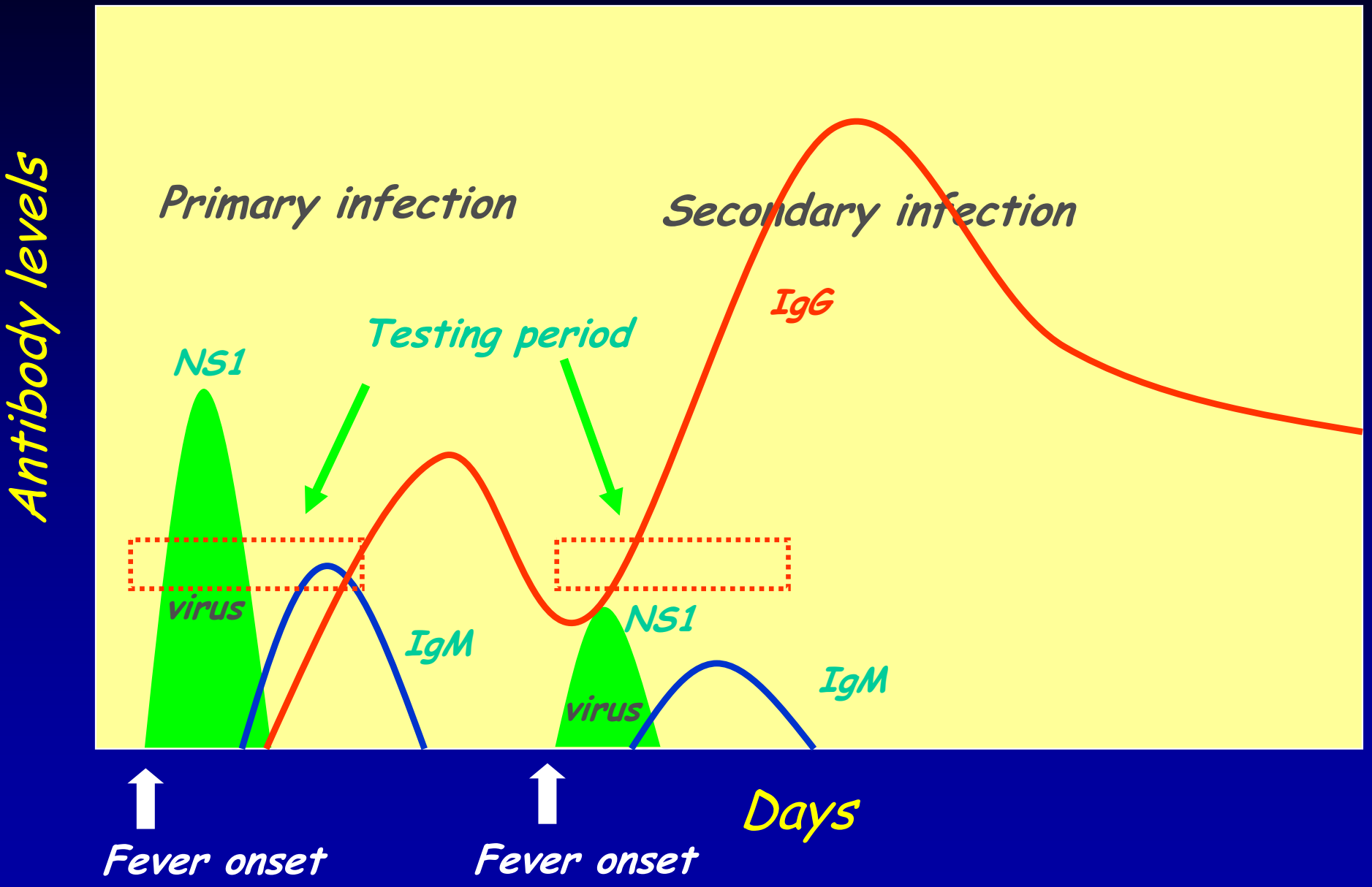
Ideal POC tests are:

- Rapid, replace waiting for lab results
- Validated in clinical trials
- Demonstrated to improve clinical outcome
  - Accurate, differential diagnosis
  - Rapid, appropriate treatment immediate
- Cost-effective in treating patients
- Used by wide range of trained personnel
- Part of a total quality system

Test needs for Dengue vaccine and control programs

- Accurate, simple, rapid tests at POC
- Early detection of primary infection
- Distinguish primary vs. secondary infection
- Determine subtypes 1 – 4
- Test for protective antibodies
- Prediction of long-term “immune memory” to immunization

“Typical” Dengue infection course



From: Paul Young-WHO/TDR/PDVI Workshop on Dengue Diagnostics Oct 2004

Opportunities to detect primary Dengue infection

- **Early detection** - viral antigen or RNA
- **Serology** – IgM antibody >5 days or 4-fold rise in titer
- **Culture** – Virus isolation

Our current Dengue diagnostic "toolbox"

- Virus isolation
- RT- PCR or NASBA
- Plaque reduction neutralization
- Hemagglutination inhibition
- IgM, IgG, NS1 ELISA
- Simple/rapid IgM, IgG tests
  - **particle agglutination**
  - **solid-phase "dipsticks"**
  - **lateral flow tests**

Technically complex - reference laboratory-based

Relatively simple, clinical laboratory-based and/or potentially point-of-care

All current methods have deficiencies for use at POC

	Culture	PRN/HI	NAAT	ELISA	Rapid
A	-	-	-	+/-	+
S	+	+	+	+	-?
S	+	+/-	+	+	+/-?
U	-	-	-	-	+
R	-	-	-	-	+
E	-	-	-	-	+
D	-	-	-	+/-	+

(**A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid/robust, **E**quipment-free, **D**elivered)

Some Rapid Point-of-Care Tests

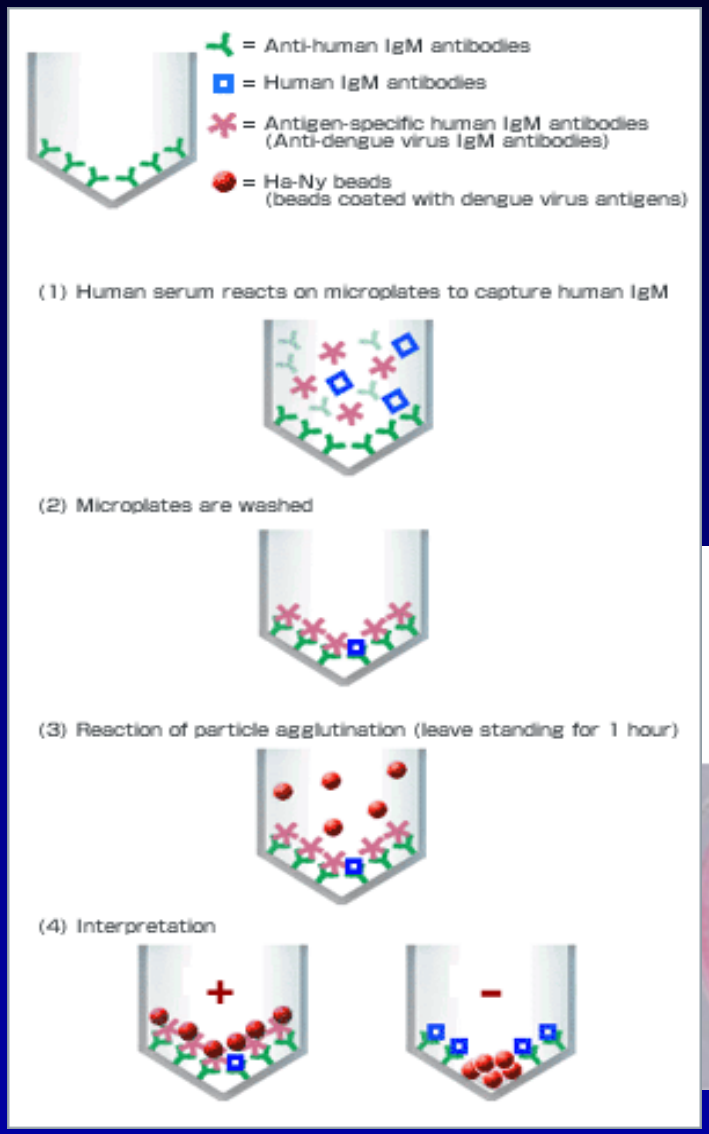
Early results were promising!

Solid-phase “dipsticks”

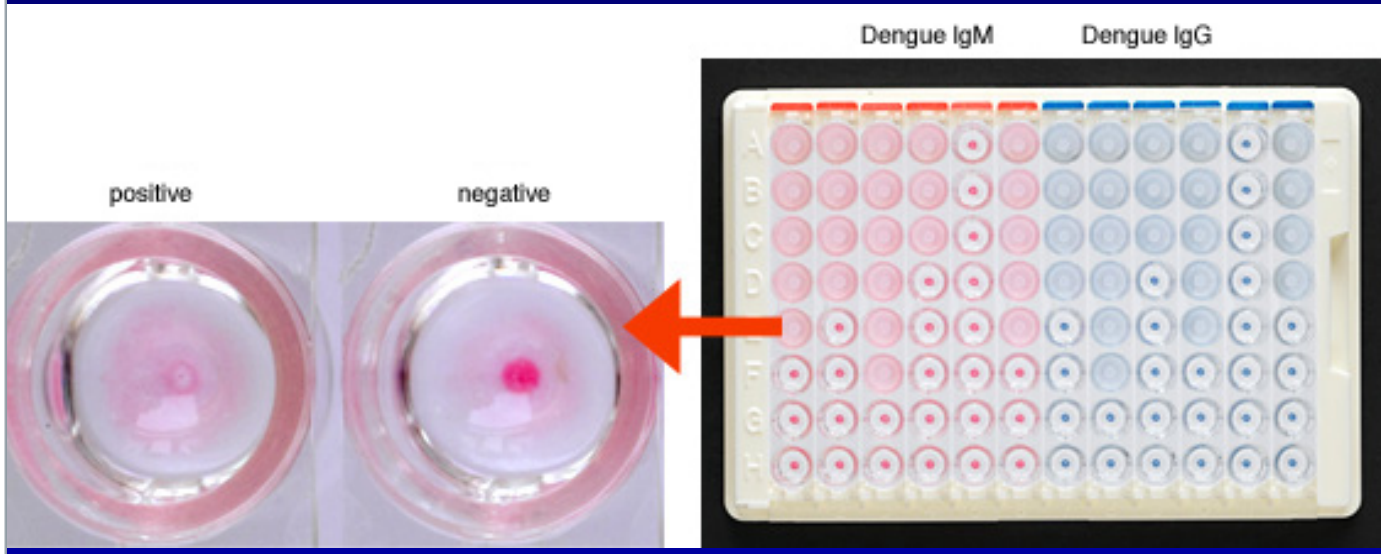
ELISA on a stick
Multi-step
Multi-analyte

PanBio
(Orgenics also)

Pentax particle agglutination test

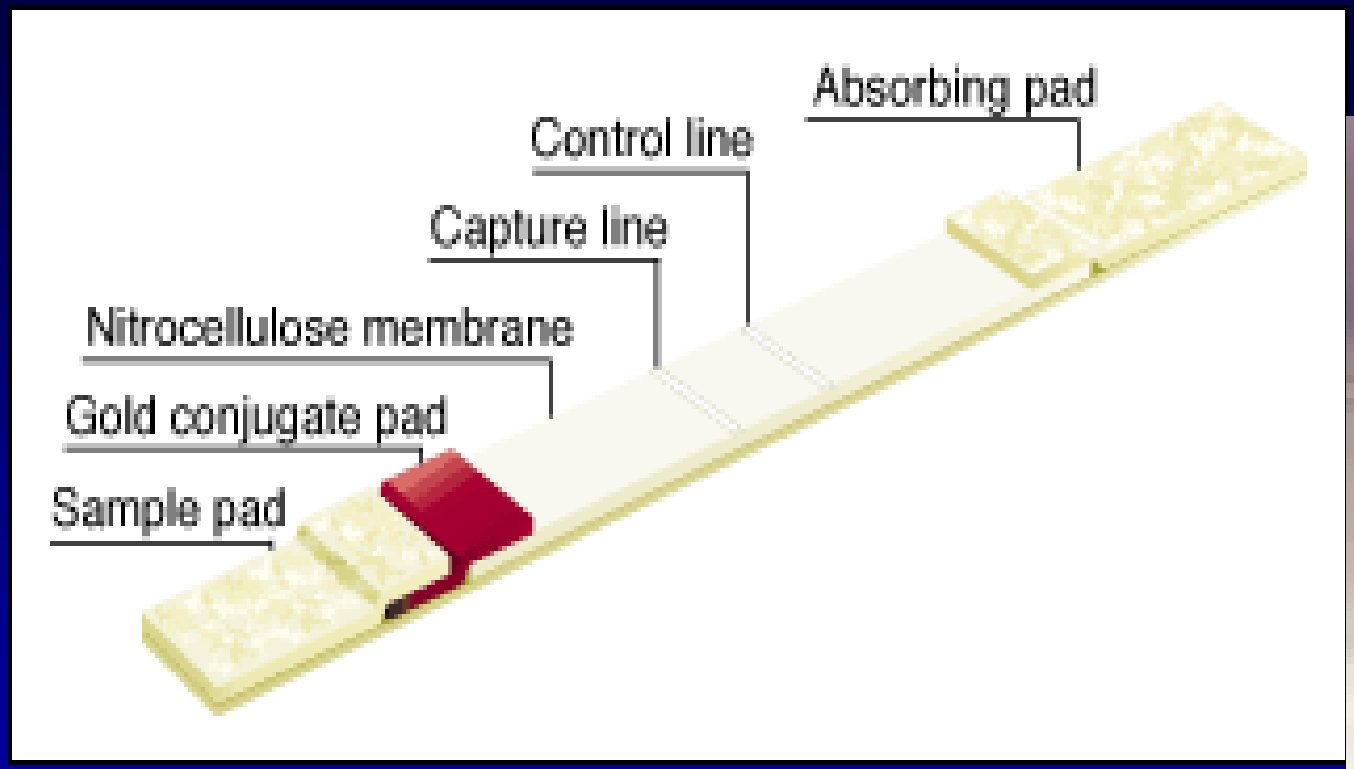


- Simple methodology
- Colored hydoxyapatite beads
- Microwell Agglutination test
- Test takes ~60 minutes



Pentax, Tokyo, Japan

Lateral flow (LF) test
(immunochromatographic strips)

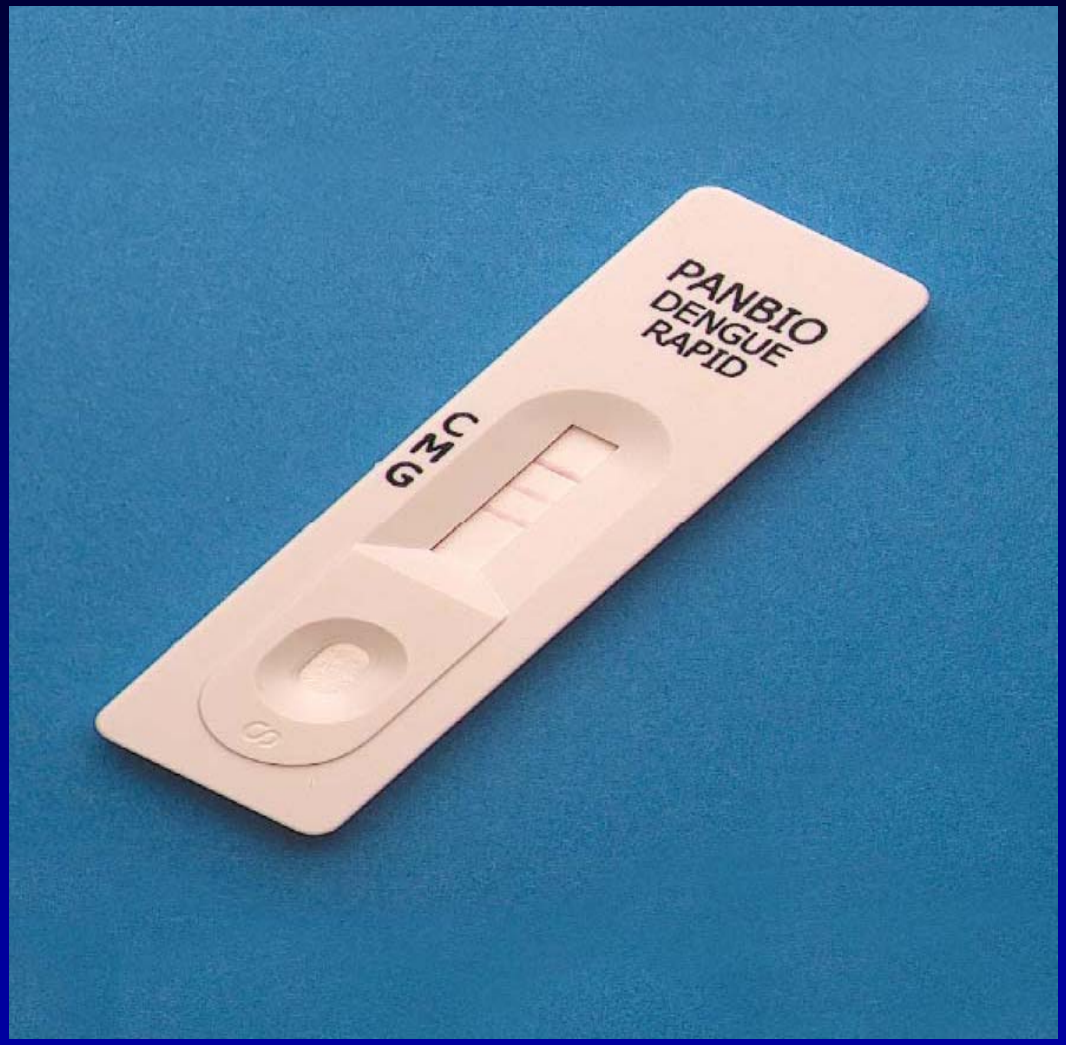


IVD News

PATH

- Simple, “Walk-away” test
- IgM and/or IgG
- Results in 5 – 10 minutes
- Initial results were promising

PanBio IgM/IgG rapid LF test



From PanBio's web site

Primary and secondary infections may be recognized by interpretation of results.

But sensitivity and/or specificity can be suboptimal

- For example, sensitivity limits of HBsAg tests:
  - ELISA 0.1 – 0.2 ng/ml
  - Rapid LF test 1 – 2 ng/ml
- Antigens or antibodies may not be dengue-specific and/or nonspecific or low-affinity antibody can lower specificity

Comparative evaluation of commercial rapid IgM LF tests

- Blacksell et al., 2006, Lab evaluation
- Blacksell et al., 2007, Prospective evaluation in Laos
- PDVI-WHO/TDR Dengue serum panel

Rapid IgM LF tests fall short of manufacturers' claims

Company	Sensitivity	Specificity
Core	22.9	98.8
Diazyme	17.8	98.2
Globalemed	62.9	69.1
Minerva	8.6	100
PanBio	65.3	97.6
Standard	21.8	98.8
Teco	9.5	97.0
Tulip	2.9	96.3

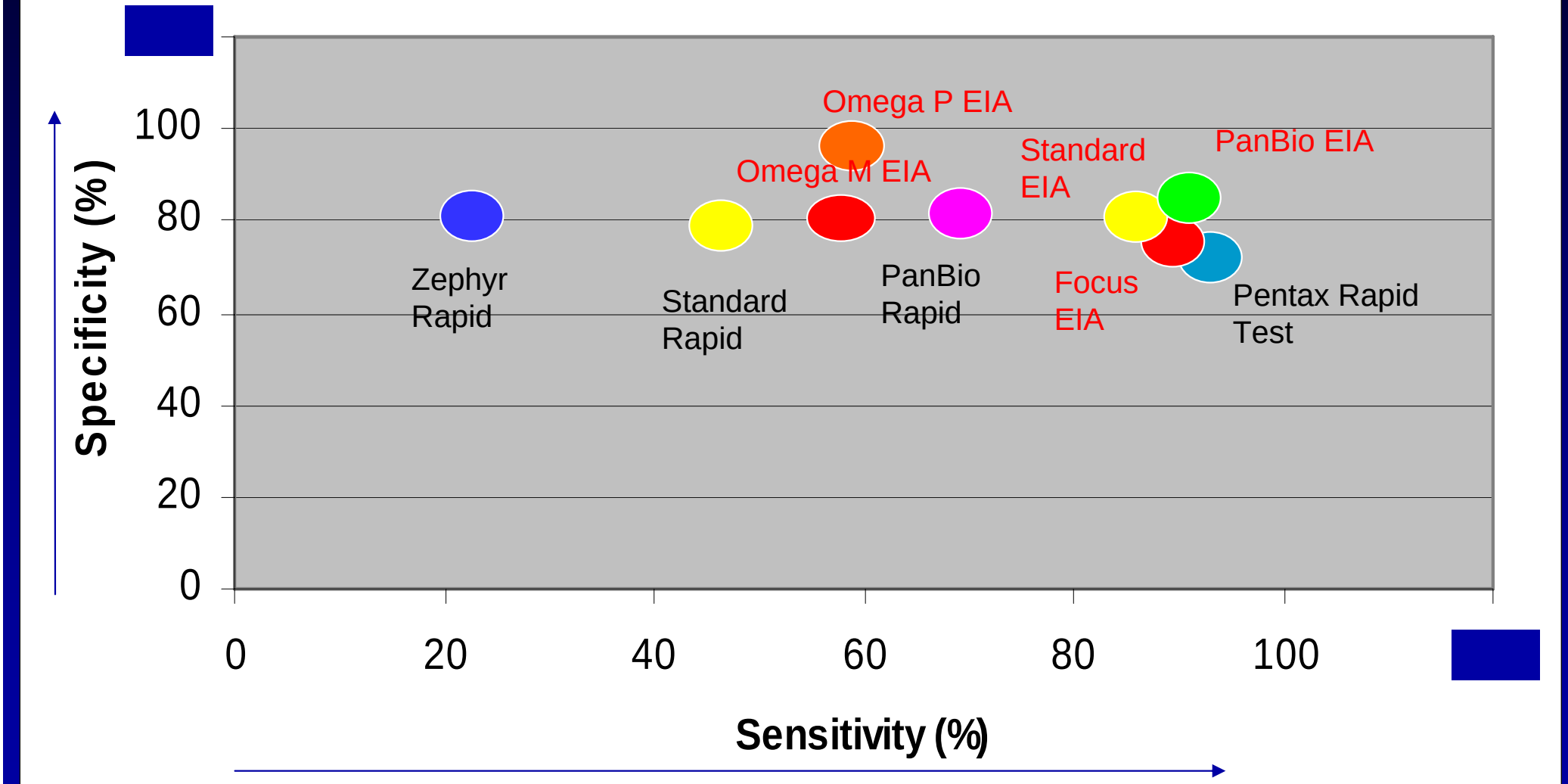
S. Blacksell et al, 2006. Laboratory evaluation of 491 positive, 491 negative sera

Rapid IgM LF tests fall short of manufacturers' claims

Company	Sensitivity	Specificity
Core	13.0	98.8
Diazyme	5.8	98.8
Globalemed	33.3	74.4
Minerva	8.6	93.9
PanBio	21.7	96.3
Standard	10.2	96.3
Teco	17.4	97.0
Tulip	6.4	99.4

S. Blacksell et al, 2007. 151 positive, 151 negative specimens
From prospective study in Laos

WHO/TDR – PVDI Dengue IgM ELISA and rapid test evaluation



Averaged results from 7 test sites

Rapid Dengue IgM LF tests

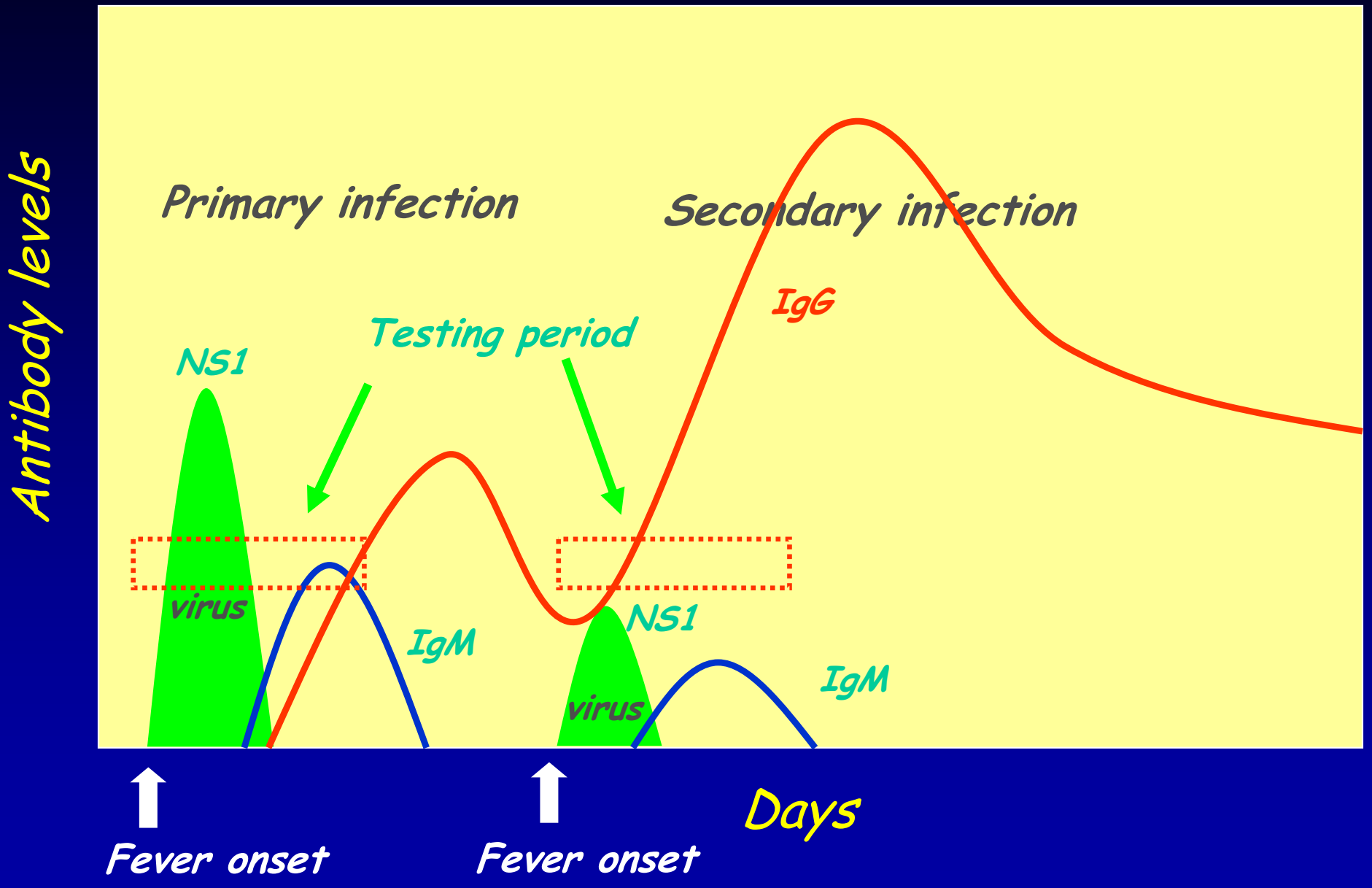
- Many commercial tests available
- All tested fell short of manufacturers' claims
- All need higher sensitivity
- Sensitivity lower during "window" period of infection
- Specificity could be better?
- Not useful for secondary infections

Why do Dengue IgM LF tests fall short of claims?

- No IVD regulation in majority of developing countries
- No manufacturing and QC standards
- Stability of many tests uncertain
- Clinical evaluation, data processing standards lacking
- Few validations of manufacturers' claims
- Reference serum panels lacking

NS1 Antigen Detection – Earlier Diagnosis of Primary Infection?

“Typical” dengue infection



From: Paul Young-WHO/TDR/PDVI Workshop on Dengue Diagnostics Oct 2004

Dengue NS1 Tests

- Detection of NS1 antigen in “window” period
- Can be positive when IgM or NAAT are negative
- Commercial ELISA include:
  - Platelia (BioRad), pan-E Dengue Early ELISA (PanBio)
- Rapid NS1 LF test – in development
  - **Publications?**
  - **Sensitivity, specificity?**
- Serotype-specific anti-NS1 antibody test?

Improvements to Current POCT

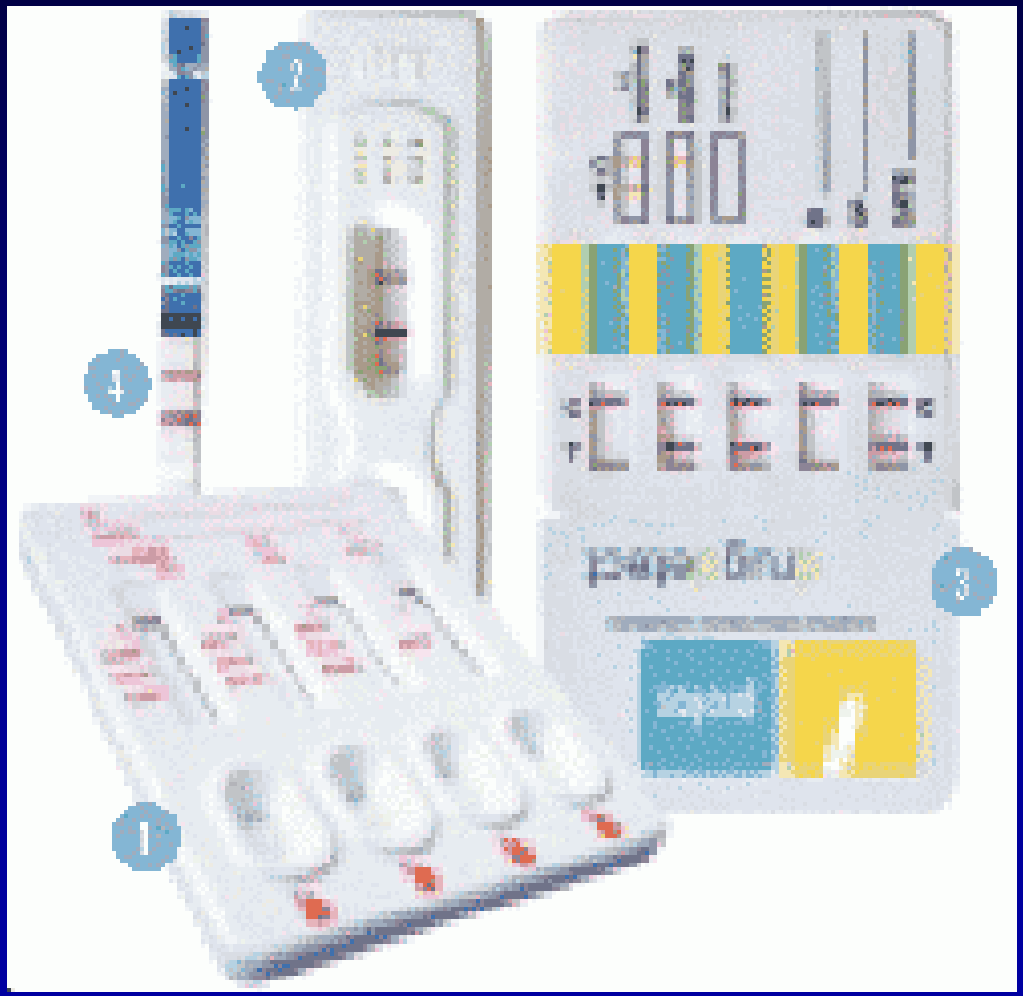
- **Sample preparation** – whole blood, dried blood spots (?), oral fluids (?), immune complexes (?)
- **Sensitivity** – higher affinity antibodies, new Mabs, aptamers, mimitopes, multi-antigenic recombinant antigens
- **Specificity** – neutralize cross-reactive Abs, use more specific antibodies, antigens
- **Materials** – Higher quality, consistency

Improvements to Current POCT

- **Manufacturing** – better protocols, QA/QC, packaging, stability, consistency
- **Better signal reagents** – fluorescent beads, paramagnetic beads, LF-EIA
- **Hand-held readers** – small, battery-powered devices, allow quantitative readout
- **Detection of PCR products** by LF
- **Combination tests** – IgM + NS1

POC tests in development

Improving Lateral Flow Tests



-Higher sensitivity and specificity

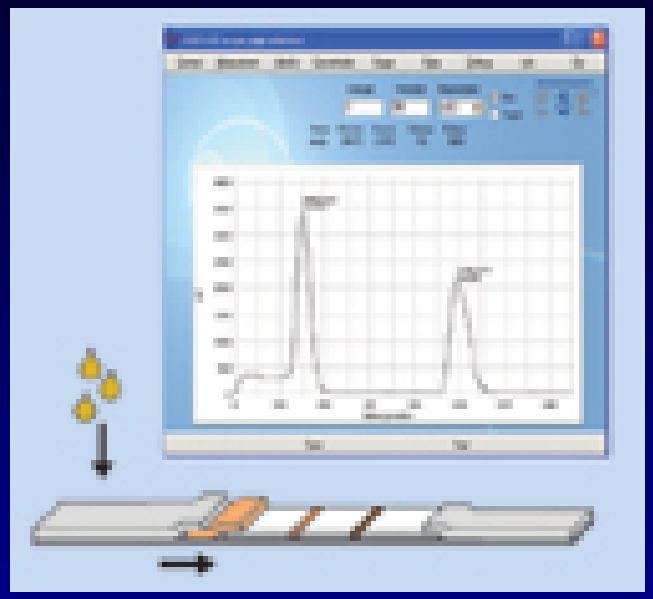
-Individual tests for serotypes 1-4?

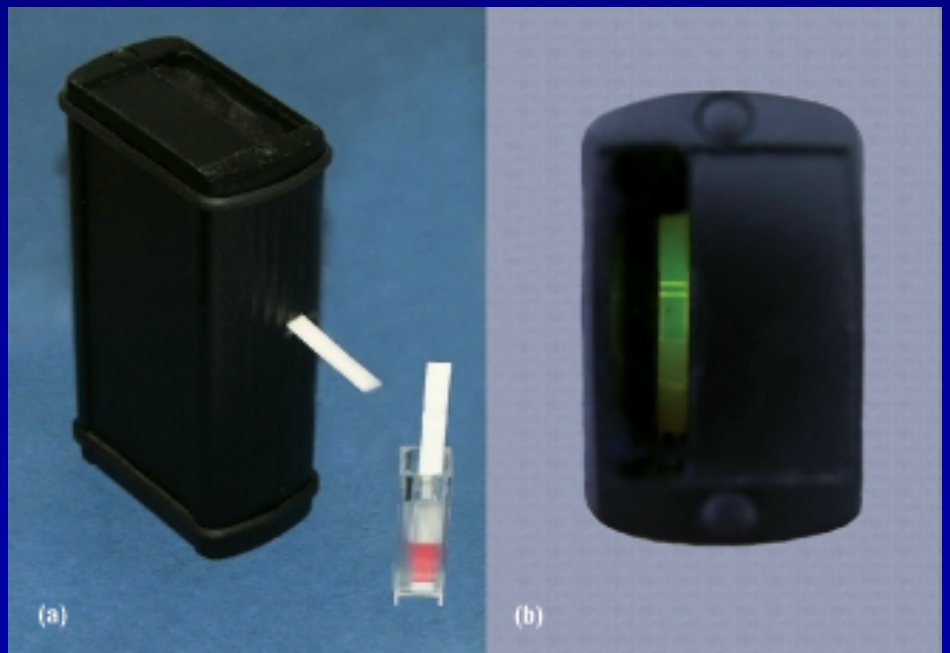
-Tests for other flaviviruses?

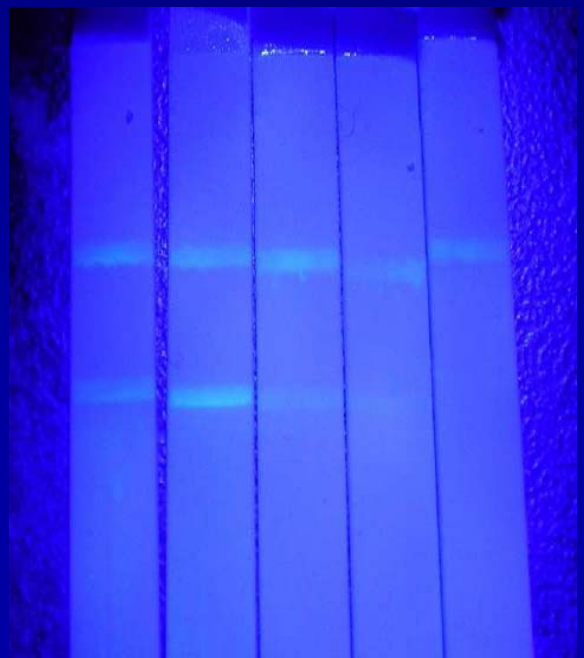
-Tests for neutral-lzing or protective antibody?

-semi-quantitative assays

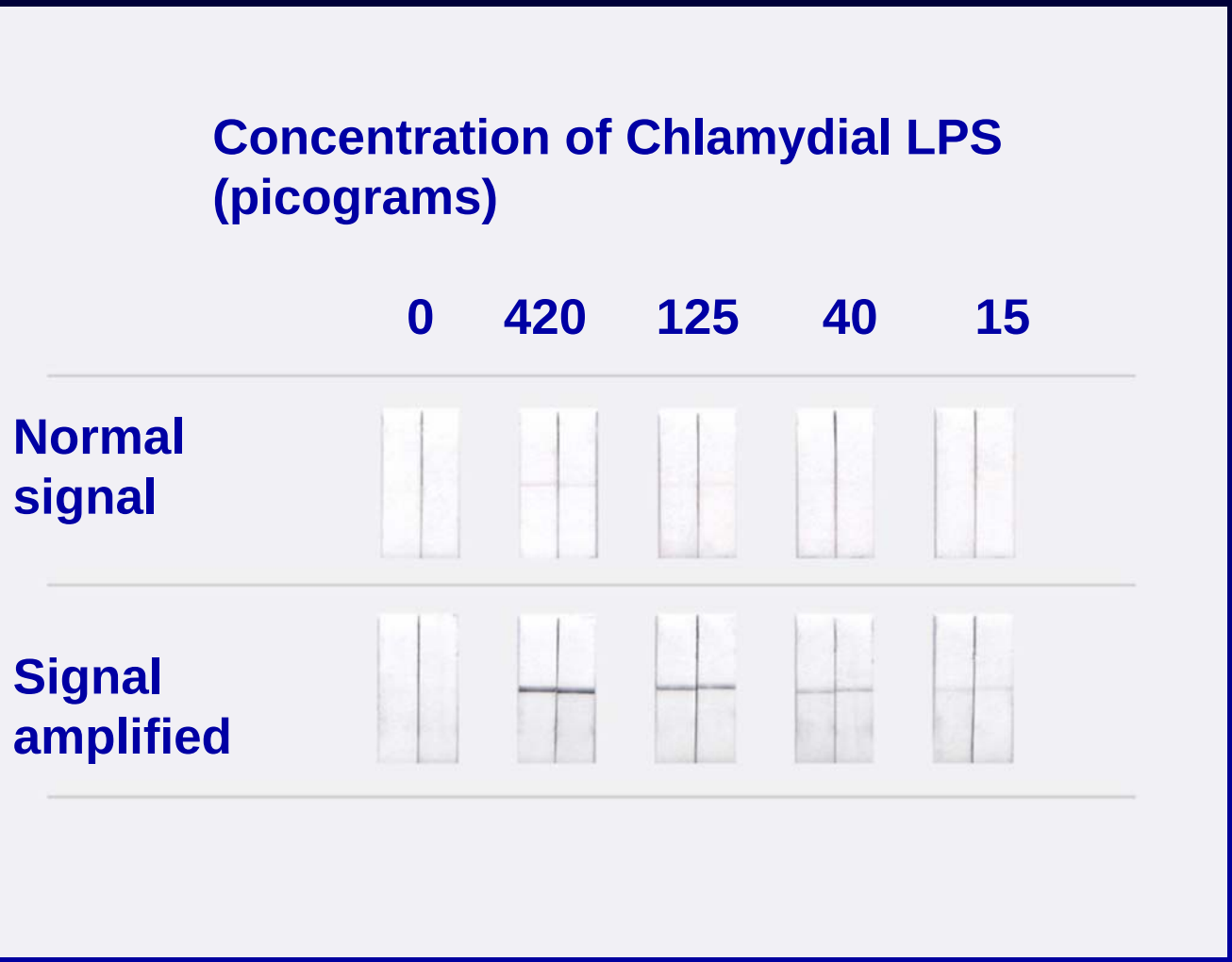
Improving Lateral Flow Tests





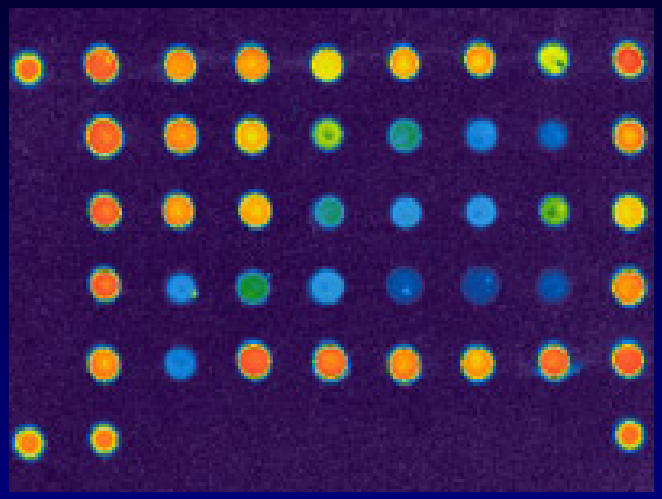


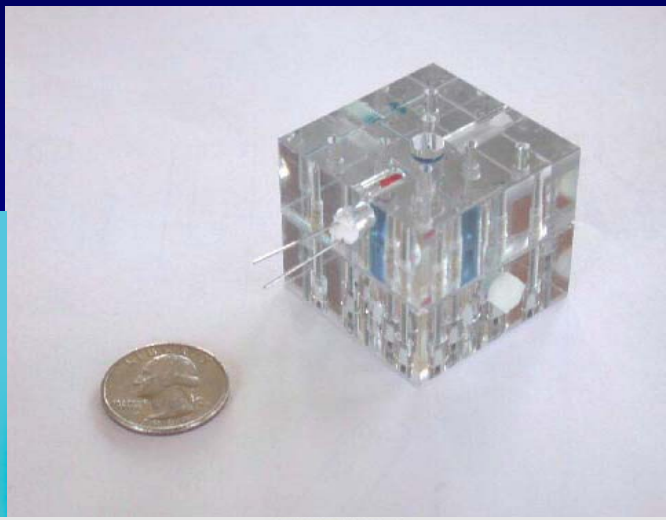
Diagnostics for the Real World

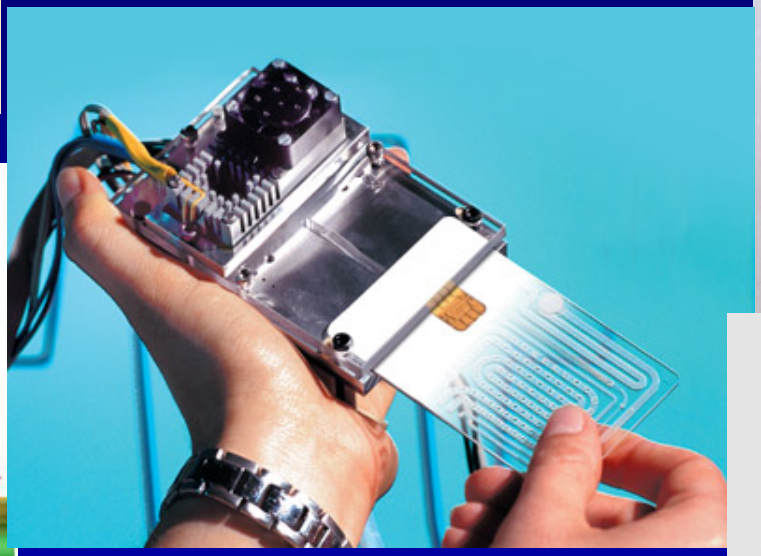


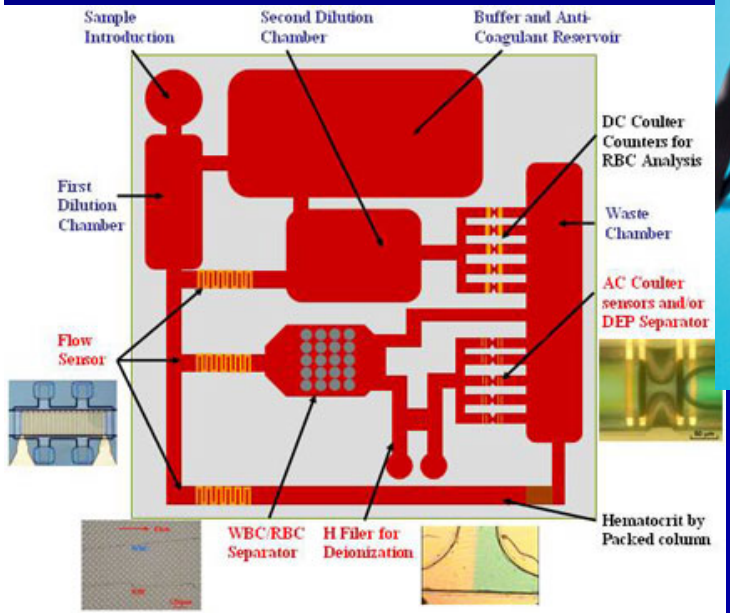
Improved antigen concentration device and “enhanced” lateral flow Test for Chlamydia – Helen Lee, Cambridge University

Biosensor tests?

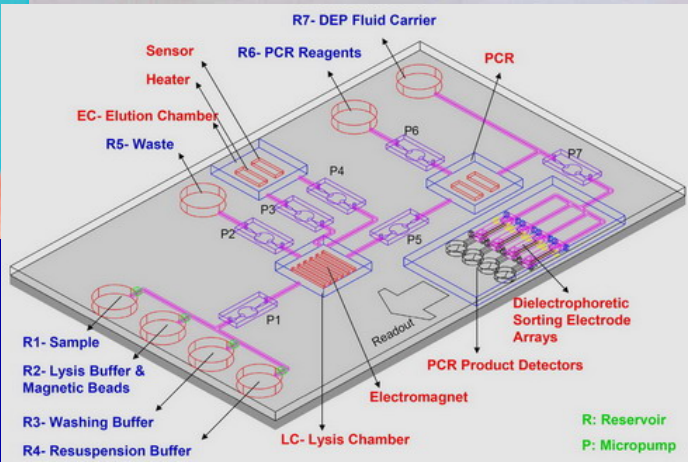








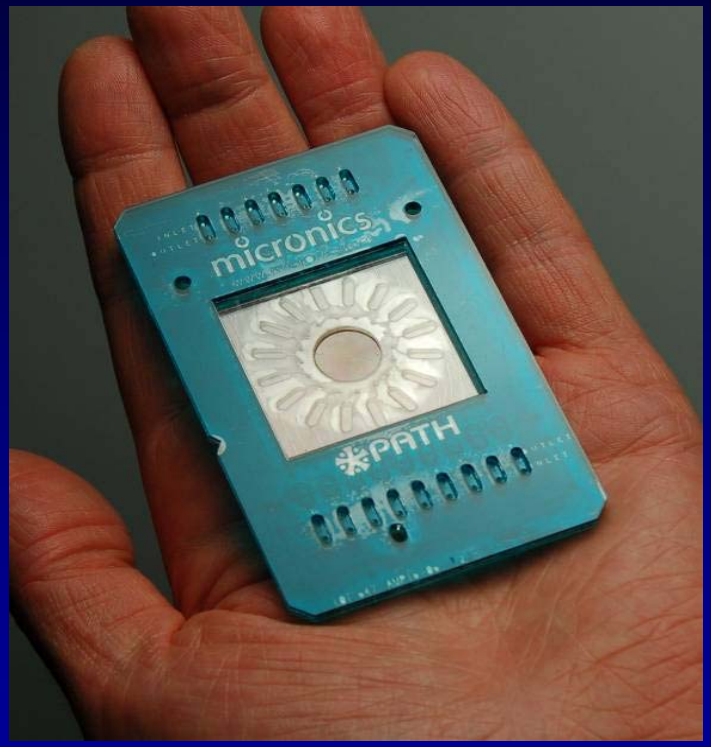
Many versions currently
In development



What biosensors can do:

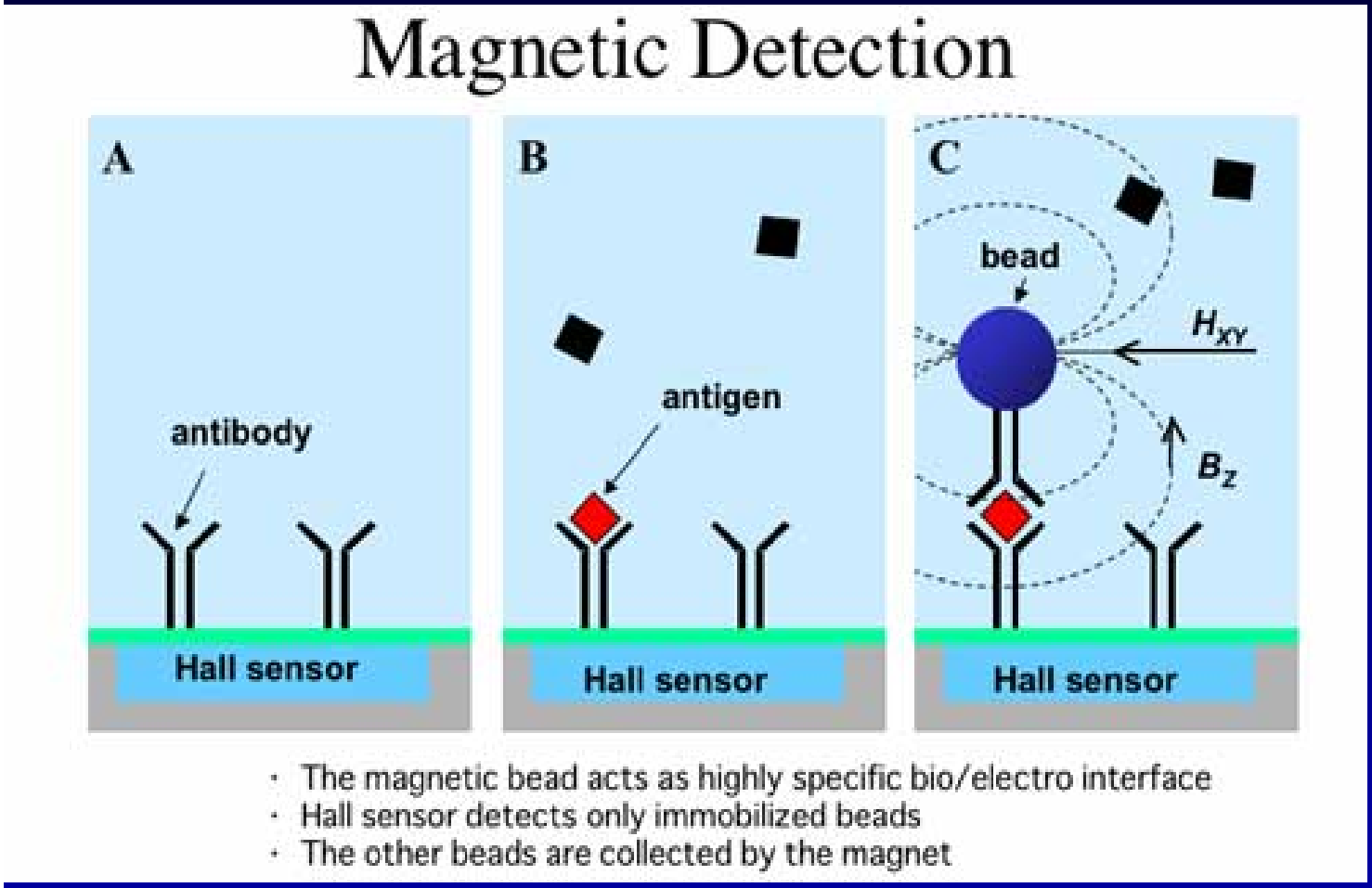
- **Integrate specimen processing, assay, read-out steps**
- **Bring NAAT and immunoassays to the POC**
- **Reduce sample and reagent volumes, and costs**
- **Reduce risk of contamination**
- **Contain waste on disposable card**
- **Reduce assay time**

PATH's "Lab on a Chip" Platform

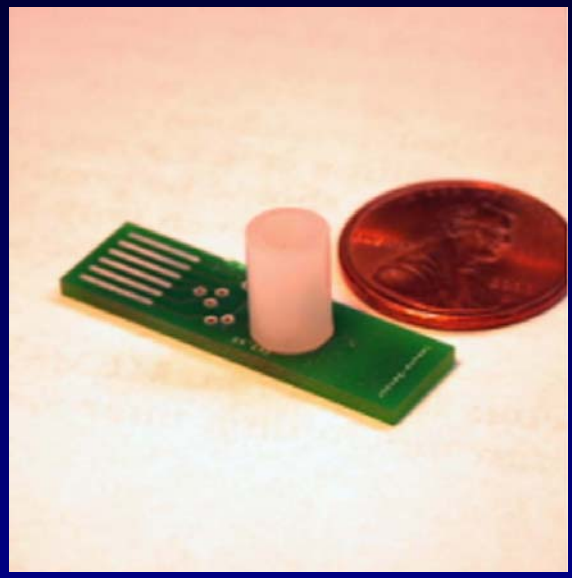


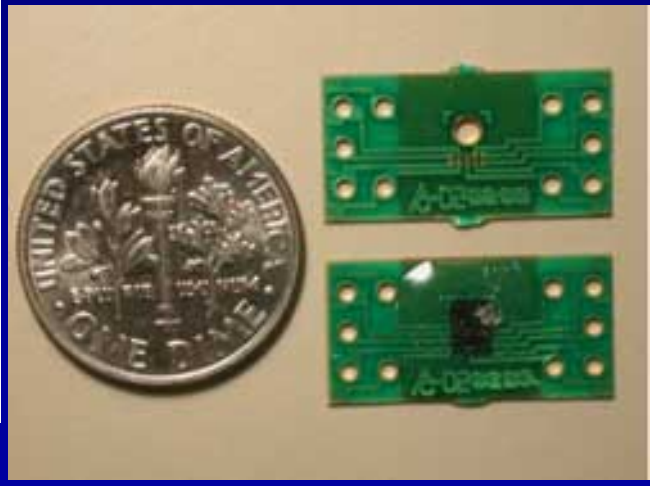
BMGF project with UW, PATH, Micronics on Fevers Panel including dengue.
From B. Weigl, PATH

UC Berkeley Magnetic Bead Sensor



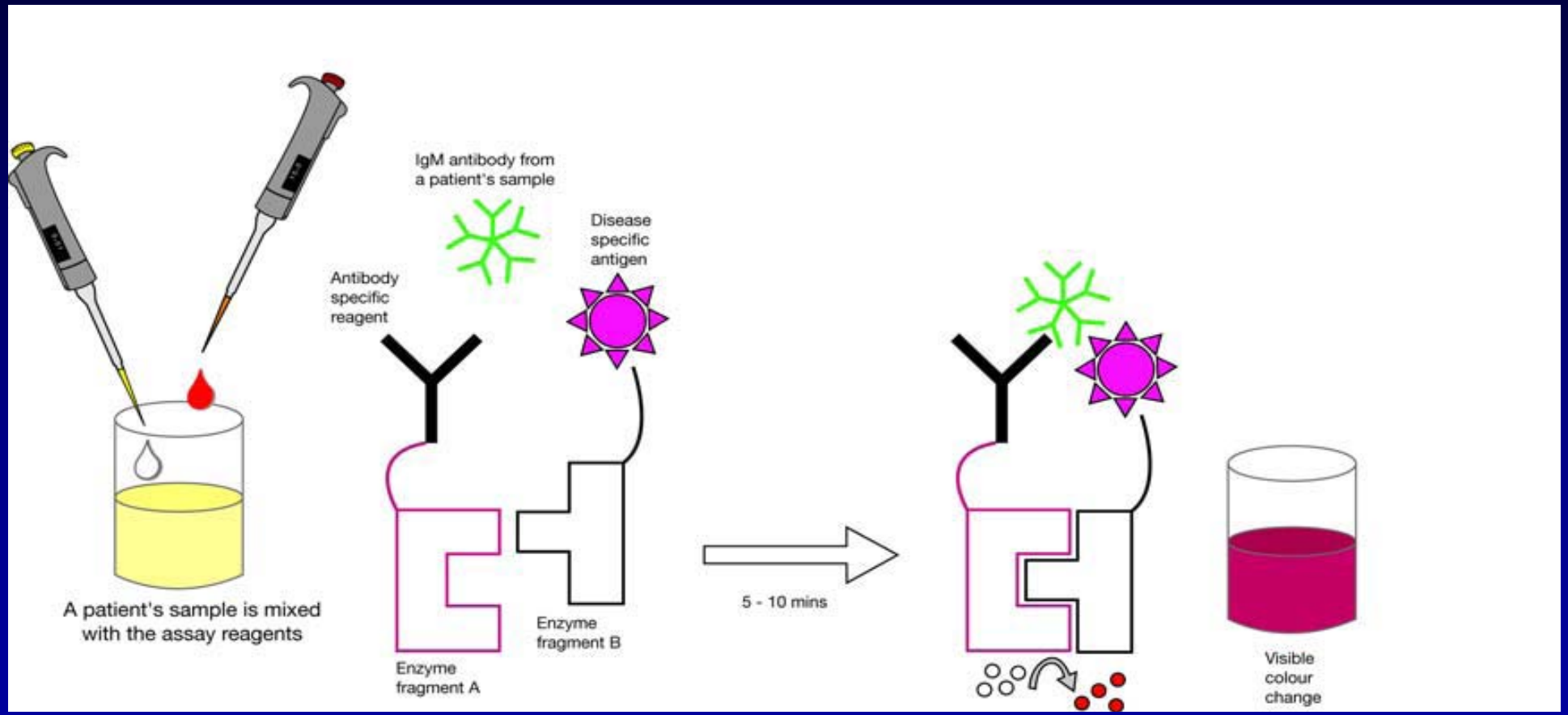
- The magnetic bead acts as highly specific bio/electro interface
- Hall sensor detects only immobilized beads
- The other beads are collected by the magnet





Homogeneous Assays

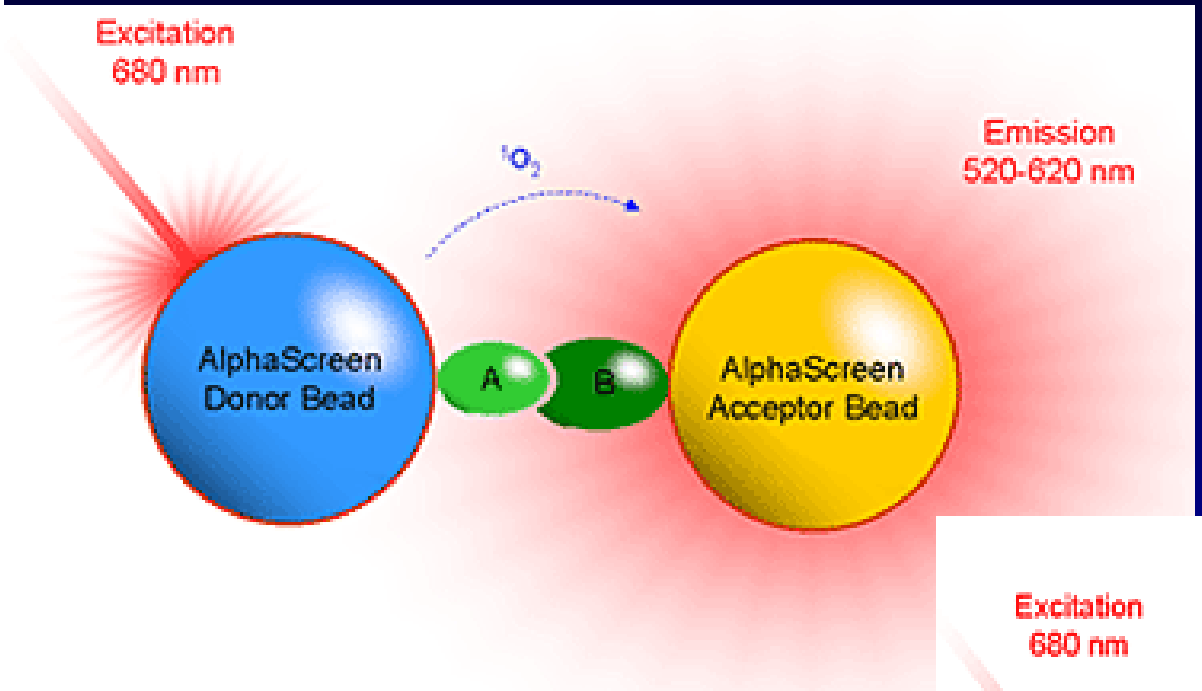
A paradigm shift for POCT?



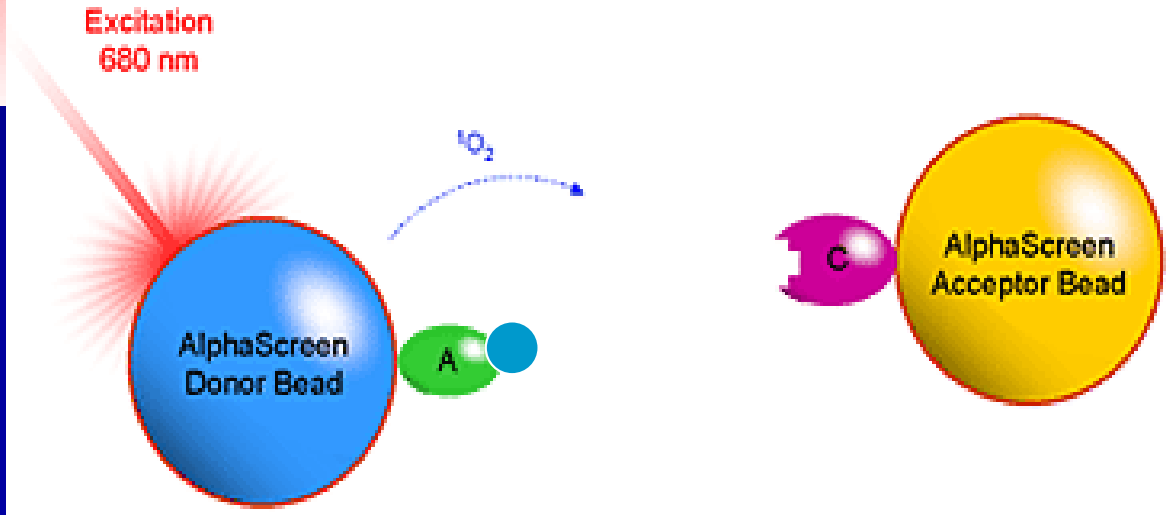
From Hazell, 2006

Kinetic assay. Colored product develops from colorless enzyme substrate.

Perkin Elmer AlphaScreen



Other examples with
Reflected light off colloidal
Gold particles



Conclusion - No optimal POC test for dengue primary infection exists right now

- **Shorter term**
  - Improve IgM rapid test sensitivity, specificity
  - Develop, validate NS1 rapid tests
  - Combine improved IgM + NS1 tests?

Conclusion - No optimal POC test for dengue primary infection exists right now

- **Longer term**
  - Develop POC and lab-based tests for neutralizing antibody, vaccine vs. wild type immunity, subtyping, secondary infection, and DSS/DHF
  - Monitor, promote development of biosensors and new technologies such as homogeneous assays

What we need to do

- **Continue to work with government labs, NGOs, and academia**
- **Engage and support industry**:
  - Develop/sustain collaborations
  - Provide reagents, specimen panels
  - Assist product development

What we need to do

- **Engage and support industry** (continued):
  - IP assistance: patents and licensing
  - Continue to provide comparative lab and field evaluations
  - Promote end-user acceptance
  - Identify and/or create markets

Questions / Discussion