



**Commercial assays for the diagnosis of acute
dengue infection –
The South-east Asian Experience**

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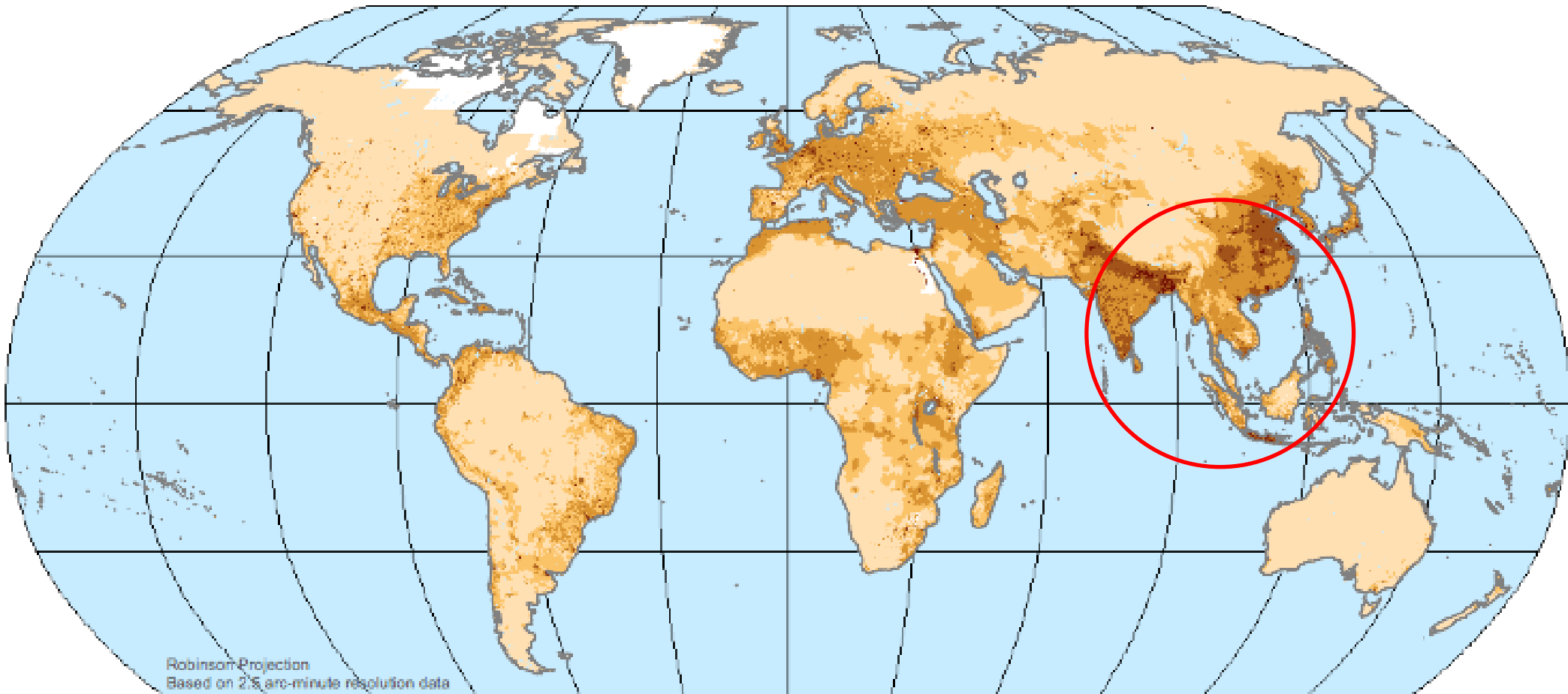
WHO dengue diagnosis expert group member

Mahidol-Oxford Tropical Medicine Research Unit (MORU) - AIM

“To develop effective, practical means of diagnosing and treating tropical infections responsible for significant morbidity and mortality in the populous rural areas of Southeast Asia and elsewhere”

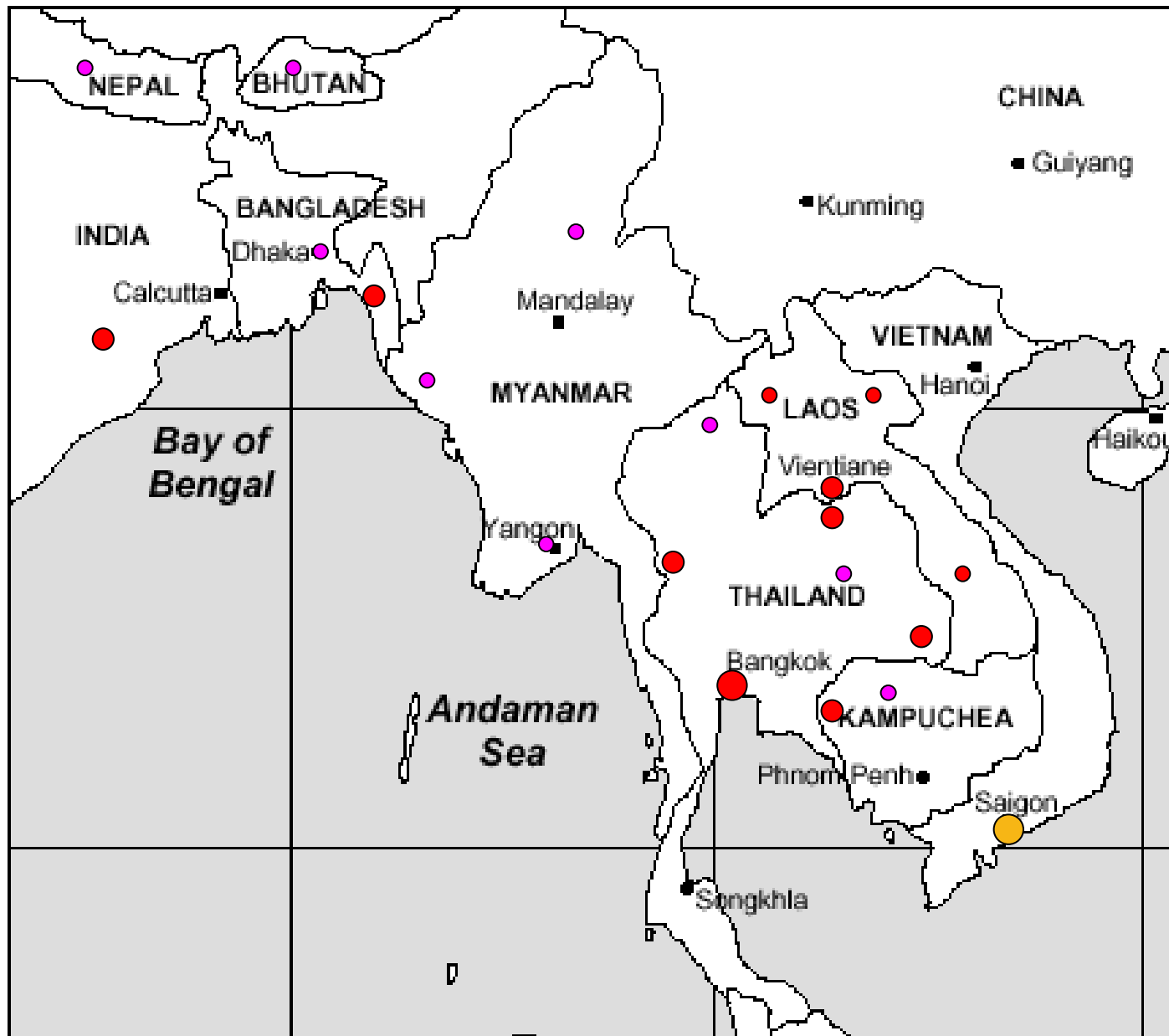


MORU's strategic position....



Half of the world's population within 3000 km....

MORU - 2009



- Bangkok Unit
- Major field site / lab
- Laos project field site
- Collaborator's site

Mozambique
 AQUAMAT collaborative s
 Sri Lanka

Mahidol-Oxford Tropical Medical Research Unit (MORU)

Locations

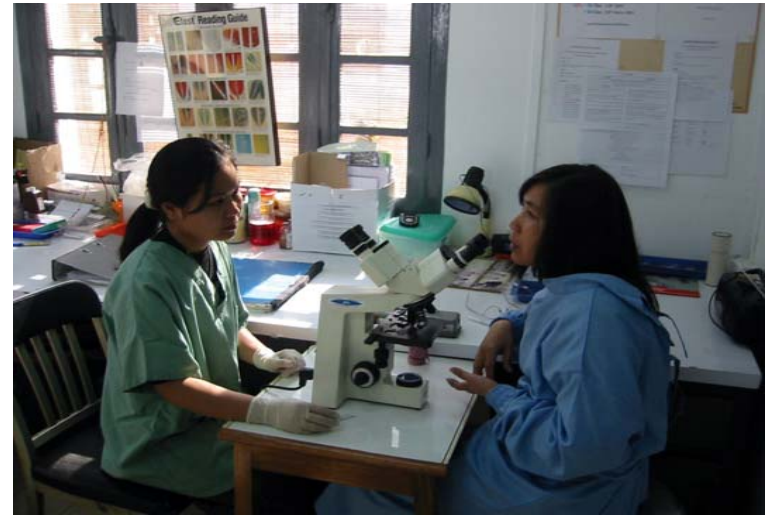
- Thailand (established early 1980's)
 - Bangkok
 - Udon Thani/Ubon Ratchathani (NE)
 - Mae Sot (West)
- Lao PDR
- Cambodia, Sri Lanka, Indonesia

Diseases studied

- Dengue
- Rickettsiosis, Malaria, Melioidosis, Leptospirosis

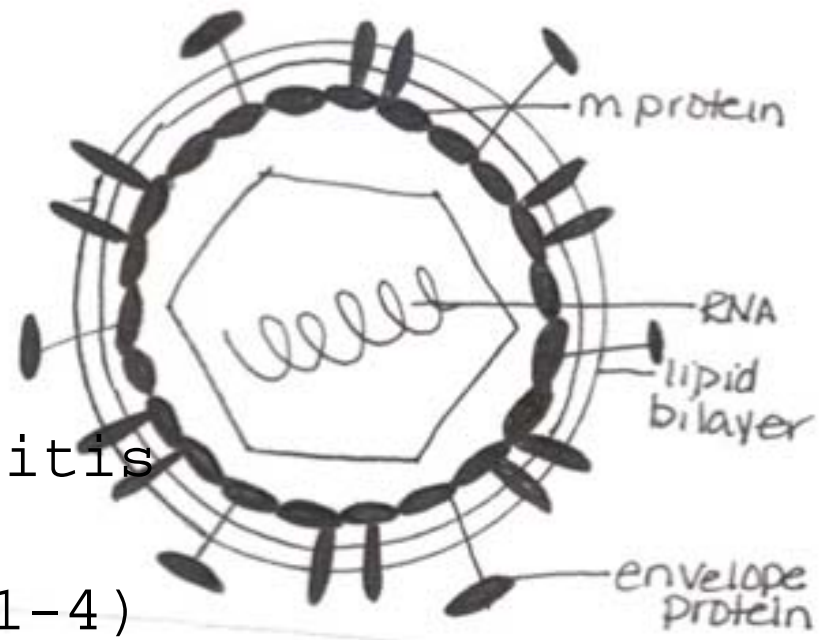
Studies

- Diagnosis & Treatment
- Diagnostic development
- Pathogenesis
- Epidemiology
- Molecular characterisation



Dengue Virus

- Member of the family *Flaviviridae*
- 3 antigenic complexes
 - Dengue
 - JE
 - Tick-borne encephalitis
- Four serotypes (Dengue 1-4)
 - All pathogenic



Clinical Dengue

- Millions of people at risk
- Clinical syndromes
 - Dengue Fever (DF)
 - Dengue Haemorrhagic fever (DHF)
 - Dengue Shock Syndrome (DSS)
- Dengue Infections
 - Primary Dengue
 - Secondary Dengue
 - Infection with a different serotypes
 - Primary risk factor for DHF/DSS
- Lab diagnosis required as dengue is clinically similar to many other tropical fevers



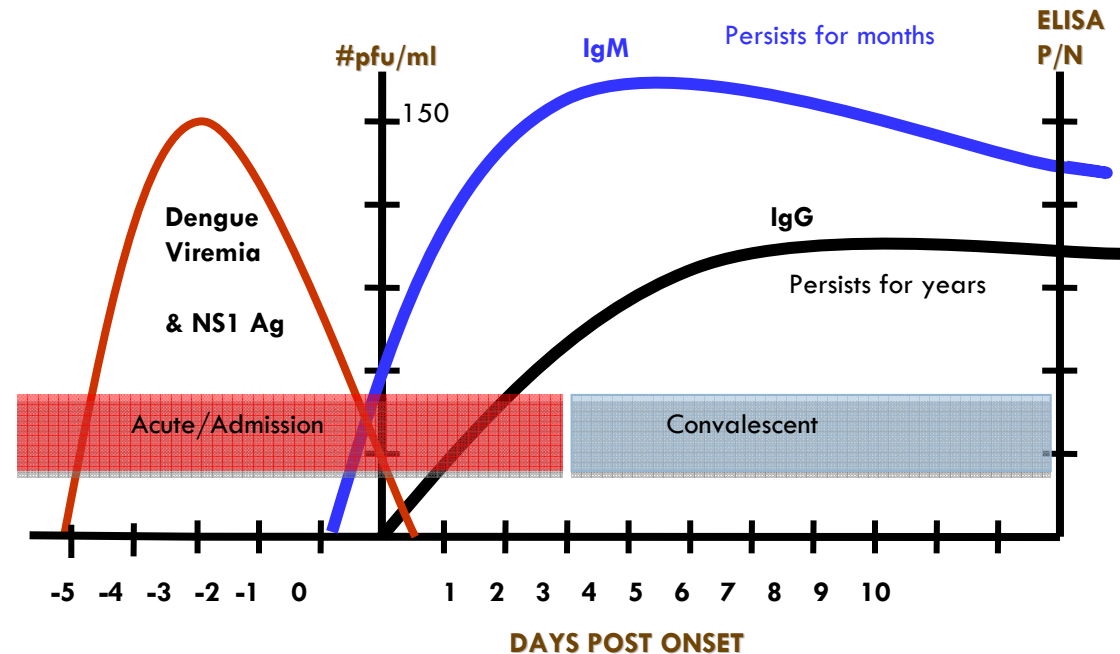
Dengue diagnostic challenge

- If we can diagnose we can treat or manage the patients
- Many tropical fevers have similar clinical presentation (Broad differential Dx)
- We need a diagnosis on presentation or soon after
- Many dengue endemic settings have limited laboratory infrastructure with limited staff training
- In rural SEA, dengue patients are treated as outpatients – Can we predict DHF?



Acute Dengue diagnosis

- Diagnosis on presentation to a health care facility
 - Clinic, Hospital, Outpatients Dept
 - Days 1-7 post-onset of symptoms
- Problems
 - Many tropical fevers have similar clinical presentation (Broad differential Dx)
 - **Sample timing is critical**
 - Rural settings
 - Limited laboratory infrastructure
 - limited staff training –
 - cost may be an issue
- Need
 - Accurate, simple and affordable diagnostic tests

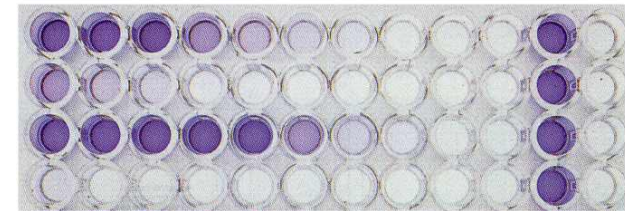
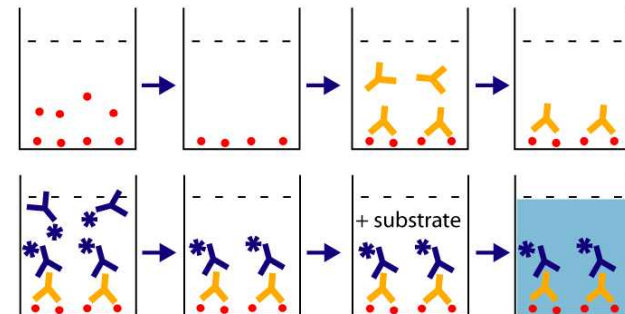
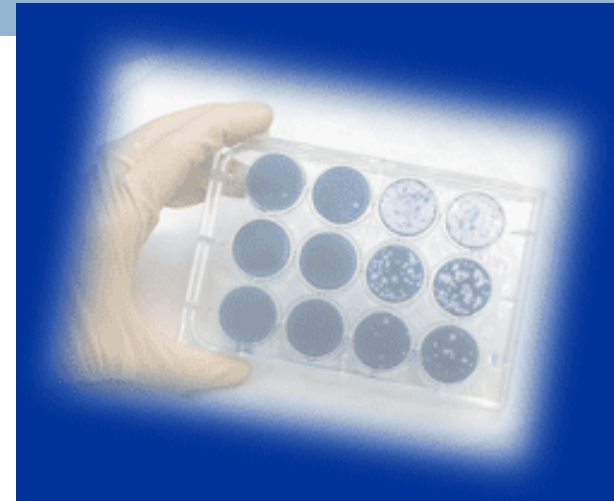


Today's message

- Sample timing (days of fever) is critical for the choice of test
 - ▣ Impact on assay sensitivity
- Know what diagnosis do you require
 - ▣ Acute dengue fever (yes/no?)
 - ▣ Primary or secondary dengue?
- Consider the setting before selecting the assay
 - ▣ ELISA or rapid test
- Combine the antibody and antigen results to improve sensitivity

Reference Dengue serology

- WHO Gold Standard Assays
 - ▣ Plaque reduction neutralisation assay
 - ▣ Haemagglutination Inhibition assay
- Alternative Gold Standard
 - ▣ IgM Capture ELISA
- Note: All assays reference require acute and convalescent sample collections



Summary of Dengue acute diagnostics – Time, cost, ease and setting

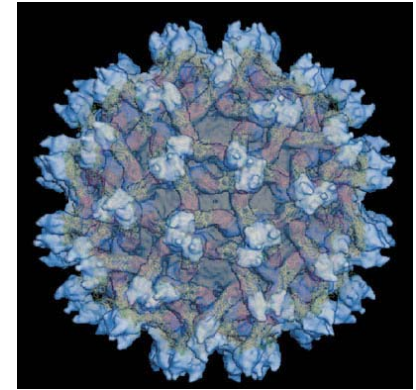
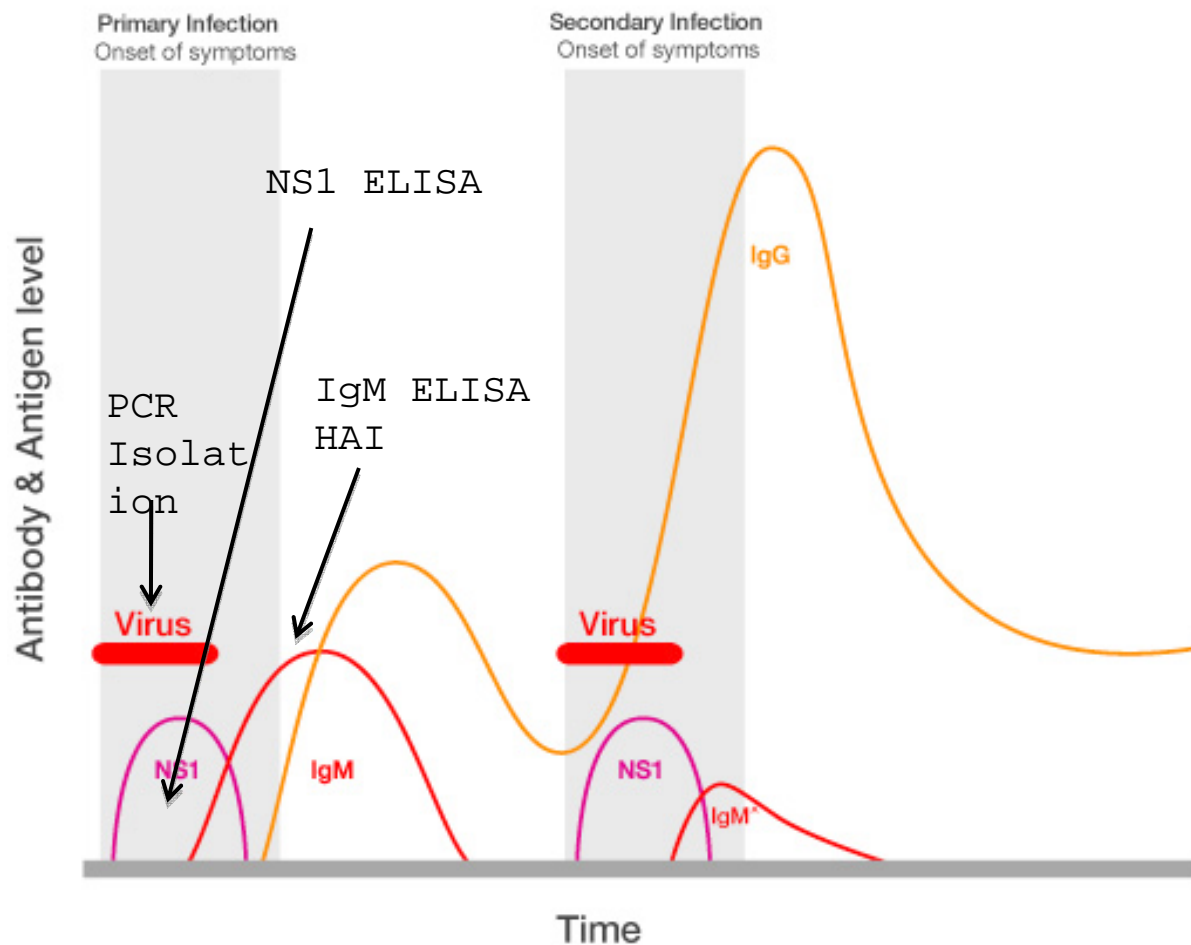
Format	Assay	Time	Ease	Setting	Cost
Serolog v	Rapid RDT	15 min	+++++	Clinic/Hosp	++
Serolog y	HAI	30 min	++++	Clinic/Hosp	+
Serolog y	ELISA	4 h	+++	Hospital/Ref lab	++
Genetic	Real-time PCR	3 h	+++	Ref lab/Hosp	++++
Isolation	Cell culture/Mosq inoculation	Days	+	Ref lab	++++

Summary of Dengue acute diagnostics – Accuracy and timing

Format	Assay	Target	Optimal timing	Primary/Secondary	Sens	Spec
Serology	Rapid RDT	IgM NS1	>5 days fever 1-5 days fever	<div style="background-color: #e06666; border-radius: 15px; padding: 10px; text-align: center; color: white;"> ??? Today's presentation??? </div>		
Serology	ELISA	IgM NS1	>5 days fever 1-5 days fever			
Serology	HAI	WA	Paired samples	Yes	++	++
Genetic	Real-time PCR	RNA	1-5 days fever	No	+++	+++++
Isolation	Isolation	Virus	1-5 days fever	No	++	+++++

Dynamics of Dengue Infection

Dengue infection: immune response



Commercial dengue diagnostics

- Market dominated by a few large manufacturers
 - Panbio, Biorad, SD, Focus
- Assay formats
 - ELISA, rapid ICT, latex, PCR, LAMP
 - IgM, IgG, NS1
- Cost
 - Can be very expensive compared to “in-house” tests
- Certifications
 - FDA, IVD, CE

Evaluation of commercial diagnostics

- Numerous accuracy evaluations ($n > 30$)
 - What is the true accuracy of an assay?
 - Be wary of manufacturers claims
 - Methodological flaws in accuracy studies
 - Difficult to compare between studies

- Situation better than a few years ago!!
 - Fewer “backyard” manufacturers
 - Less “badge engineering”

Difficult to compare the accuracy studies

1. Reference assay comparators

- Comparison with other assays (inflated sensitivity)
- Final patient diagnosis is the most realistic comparator

2. Sample timing

- Early acute (admission)
- Late acute (day 7-10 post-admission)

3. Patient demographics

- Geographical location (endemic?)
- Age
- Immune status

4. Sample Recruitment method

- Prospective
- Case-control

Blacksell *et al*, 2006. *TRSTMH* 100: 775

Dengue ELISA evaluations

ELISAs for acute dengue Dx

- Formats – 96 well MTP replaced Dot Blots
- ELISA market dominated by a few manufacturers
- Applications
 - ▣ IgM and NS1 ELISAs – acute Dx
 - ▣ IgM and IgG ELISA - acute Dx and infection status
- Sample timing is critical for accurate Dx
 - ▣ Patient recall is a problem
- Cross-reactions (false positives)
 - ▣ Acute scrub typhus, malaria & leptospirosis (antibody)

IgM capture ELISAs – Selected studies

Manufacturer	Author/Year	Sens**	Spec
Panbio	Cuzzubbo et al - 1999	74%	100%
	Groen et al - 2000	87%	96%
	Cuzzubbo et al - 2000	95%	100%
	Lam et al - 2000	95%	94%
	Vazquez et al - 2007	97%	>99%
	Blacksell et al – 2008 – ACUTE samples	51%*	84%
MRL (Focus)	Porter et al – 1999 – ACUTE samples	38%*	94%
	Branch et al - 1999	98%	100%
	Palmer et al - 1999	98%	100%

* Acute samples only

**Samples mostly mixed acute and convalescent timing

Panbio Duo ELISA – Selected studies

- Dengue IgM and IgG capture ELISA
 - Recombinant antigens
 - Differentiate primary and secondary infections using high titer IgG responds and presence/absence of IgM

Author/Year	Acute specimens		Correct assignment	
	Sensitivity	Specificity	Primary	Secondary
Sang et al - 1998	55%	96%	88%	96%
Lam et al - 1998	97%**	89%	100%	97%
Porter et al - 1999	4% (med: 4 DOF)	94%	63%	100%
Vaughn et al - 1999	79%	92%	100%	96%
Cuzzubbo et al -2000	95%**	100%	ND	ND

** sample timing not stated

2007 - Can NS1 Antigen help?

- Using the same samples from Mahosot Hospital, Vientiane, Laos (2005-2006)



Available online at www.sciencedirect.com



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DIAGNOSTIC
MICROBIOLOGY
AND INFECTIOUS
DISEASE

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Virology

Evaluation of the Panbio dengue virus nonstructural 1 antigen detection and immunoglobulin M antibody enzyme-linked immunosorbent assays for the diagnosis of acute dengue infections in Laos[☆]

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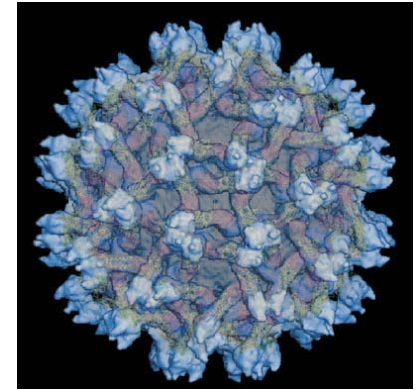
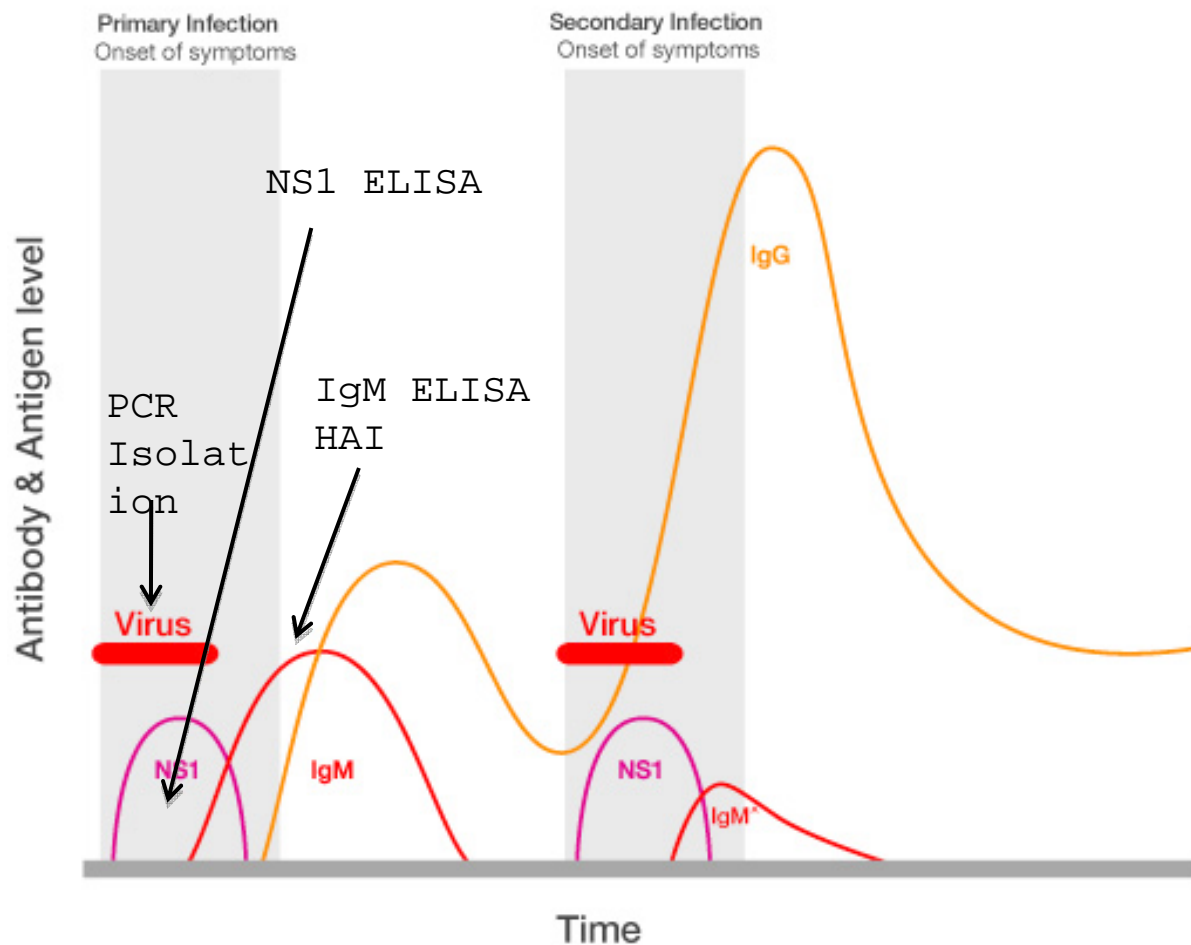
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Dynamics of Dengue Infection

Dengue infection: immune response

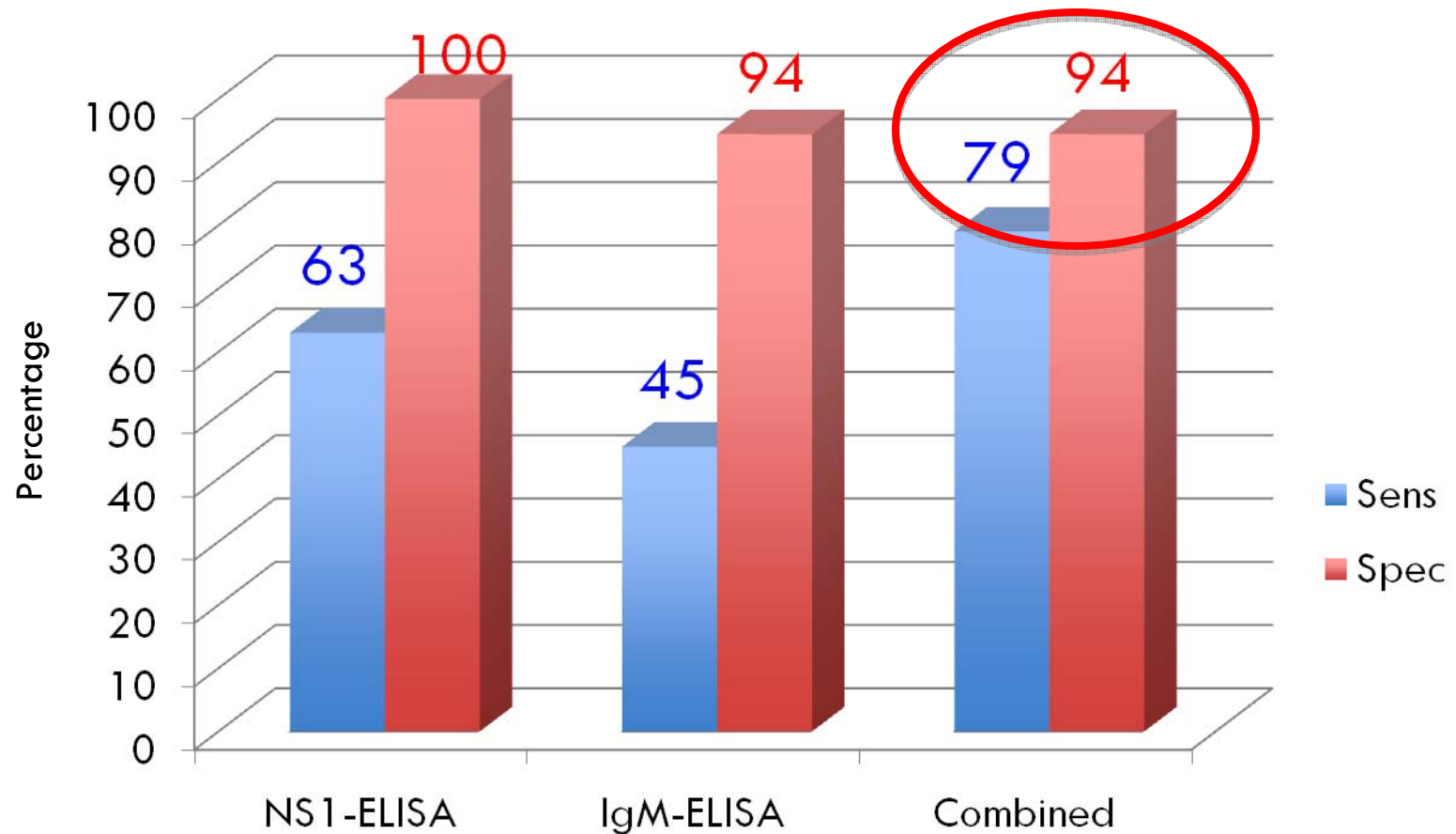


NS1 antigen detection ELISA

Manufacturer	Assay	Author/Year	Sample Timing	Sens	Location
Panbio	Pan-E	Blacksell et al 2008	Median: 5 days (IQR: 4-7)	63%	Laos
		Dussart et al 2008	82% < 5 days fever	60%	French Guiana
		Devi Sekaran et al 2008	IgM negative	92%	Malaysia
Biorad	Platelia	Bessoff et al 2008	1-5 days fever	83%	Puerto Rico
		Dussart et al 2008	82% < 5 days fever	87%	French Guiana
		Lapphra et al 2008	1-5 days fever	63%	Thailand
		Chuansumrit et al 2008	2-9 days fever	Various	Thailand
		Kumarasamy et al 2007	Not stated	93%	Malaysia
		Dussart et al 2006	0->7 days fever	89%	French Guiana

Lao cohort acute – Panbio IgM/NS1 ELISA

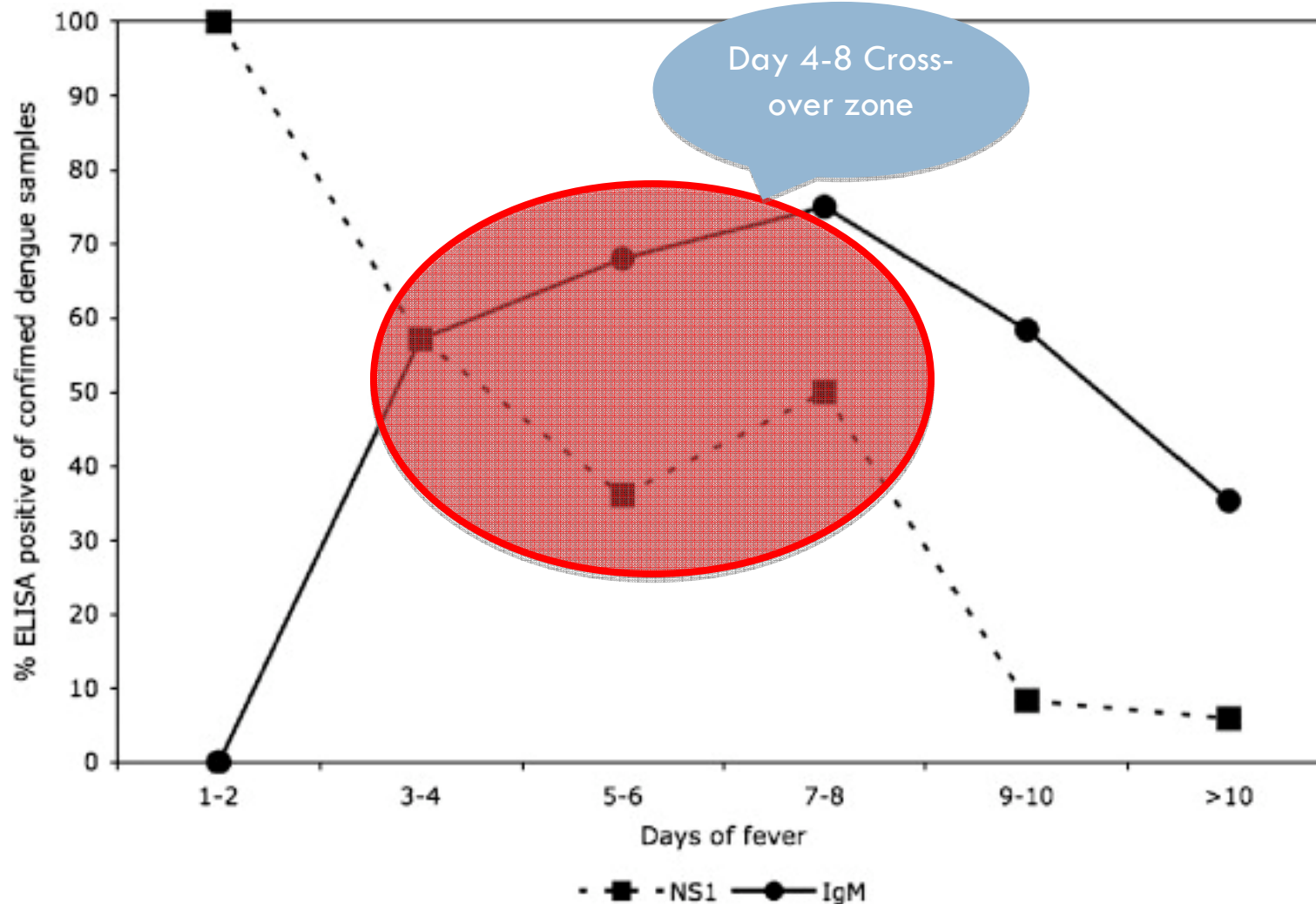
Individual and combined results



- Mahosot Hospital – Laos
- 140 pts “dengue-like” illness
- 38 confirmed dengue by gold-standard tests

Blacksell *et al*, 2008. *Diag. Micro. Inf. Dis.* 60: 45

Consider the timing of the sample- Combining of Panbio IgM and NS1 ELISA results



Blacksell *et al*, 2008. *Diag. Micro. Inf. Dis.* 60: 45

Rapid tests



POINT OF CARE TEST (POCT) EVALUATIONS

Rapid, simple, cheap POCT can improve patient outcomes in the rural tropics

No regulation = Poor POCT quality!

MORU has identified a large number of poor quality antibody-based commercial POCT for **Dengue, scrub typhus and leptospirosis** diagnosis

- Can quality and regulations be improved?
- Can combined antigen/antibody be used to improve acute diagnosis?



In Focus



The need for regulation and standardisation of *in vitro* diagnostic (IVD) assays for the diagnosis of acute tropical infections: dengue as a case study

Introduction

A recent review of diagnostics for the developing world¹ stated that the characteristics of the ideal diagnostic test were:

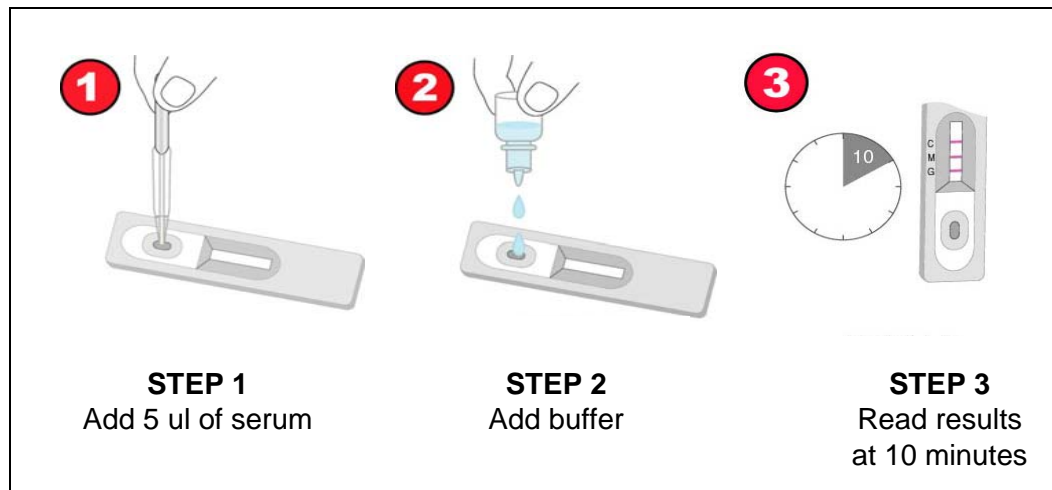
- Affordable by those at risk of infection.
- Sensitive (few false-negatives).
- Specific (few false-positives).

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labelled anti-dengue virus monoclonal antibody are dried on a pad at the head of a nitrocellulose strip which is impregnated with both anti-human IgM and IgG antibody lines (Figure 1). Test sample (serum or plasma) and running buffer are added to the pad which activates the reagents and facilitates the migration of the reagents and sample by

Dengue lateral flow procedure

Method



Interpretation



2004 -Panbio RDT meta-analysis

Transactions of the Royal Society of Tropical Medicine and Hygiene (2006) 100, 775–784



available at www.sciencedirect.com



journal homepage: www.elsevierhealth.com/journals/trst



A systematic review and meta-analysis of the diagnostic accuracy of rapid immunochromatographic assays for the detection of dengue virus IgM antibodies during acute infection

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Sharon J. Peacock^{a,b}, Nicholas P.J. Day^{a,b,c,*}, Arjen M. Dondorp^{a,b}

What is the true diagnostic
performance of the dengue rapid
tests?

Before 2004 – Dengue RDT meta-analysis

Study	Cohort Size	Dengue prevalence*	Sn (95%CI)	Sp (95%CI)
Groen et al.	54	0.09	0.45 (0.18 – 0.75)	0.97 (0.85 - 1.00)
Cuzzubbo et al.	143	0.15	0.61 (0.44 – 0.76)	0.87 (0.79 - 0.93)
Vaughn et al.	124	0.40	0.64 (0.52 - 0.74)	0.72 (0.56 - 0.83)
Kittigul and Suankeow	82	0.40	0.75 (0.59 - 0.86)	1.00 (0.91 - 1.00)
Chakravarti et al.	86	0.35	0.83 (0.67 - 0.93)	0.96 (0.85 - 0.99)
Chakravarti et al.	71	0.45	0.94 (0.79 - 0.99)	1.00 (0.91 - 1.00)
Branch and Levett	79	0.59	0.96 (0.85 - 0.99)	1.00 (0.88 - 1.00)
Lam and Devine	45	0.17	1.00 (0.78 - 1.00)	0.90 (0.72 - 0.97)
Sang et al.	69	0.22	1.00 (0.88 - 1.00)	0.57 (0.43 - 0.70)
Vajpayee et al.	59	0.69	1.00 (0.91 - 1.00)	0.67 (0.41 - 0.86)
Palmer et al.	100	0.78	1.00 (0.95 - 1.00)	0.86 (0.56 - 0.97)
Pooled (Random effects)	912	0.40	0.86 (0.74 - 0.92)	0.88 (0.78 - 0.94)
Heterogeneity (I²)			97.7%	79.6%

