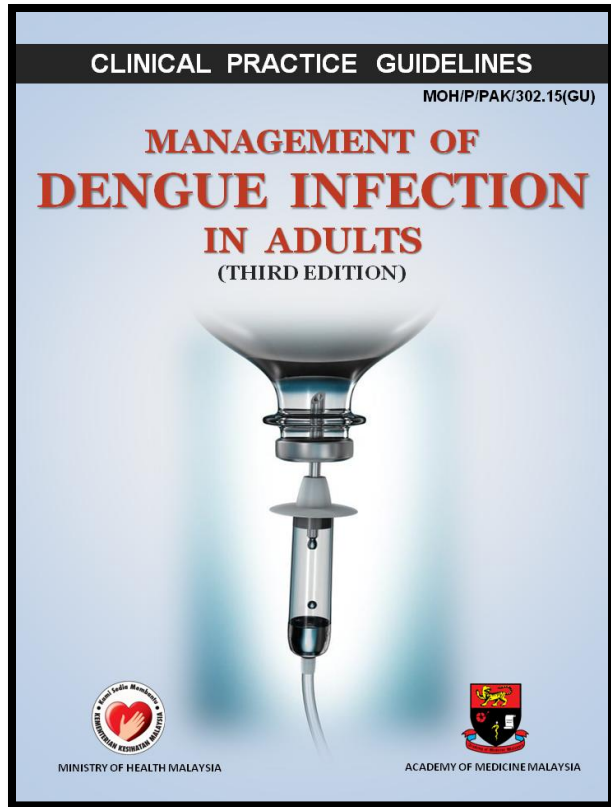




Dengue: Virology, Diagnostics & Surveillance



LEARNING OBJECTIVES

OUTLINE

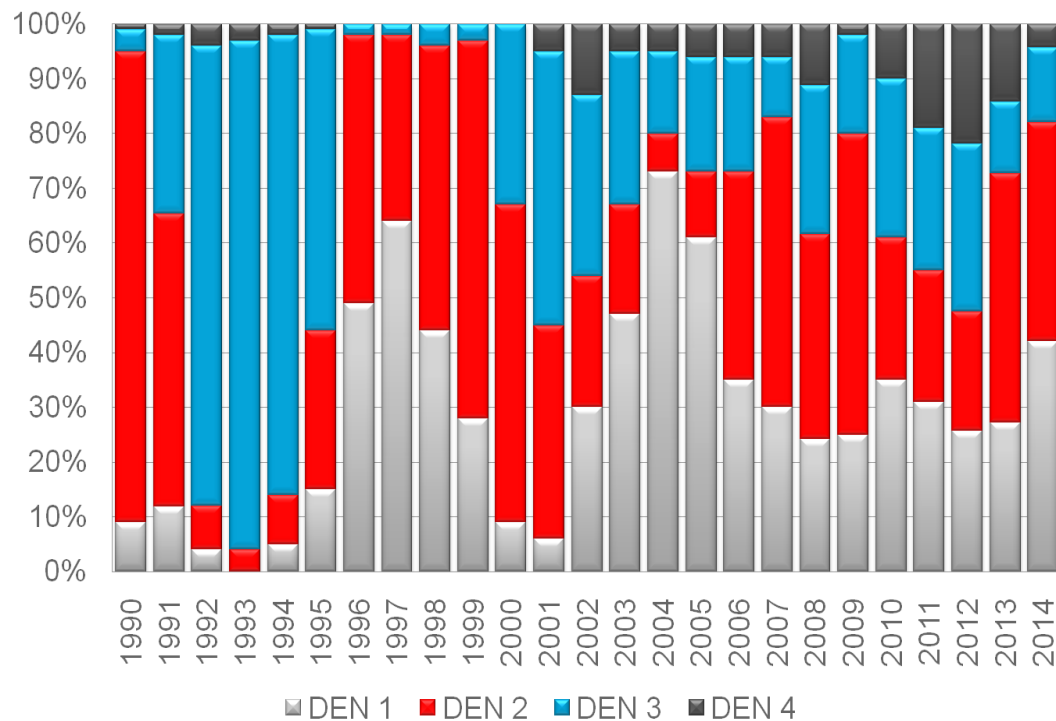
- To explain the current Dengue Virus Serotype circulation/surveillance and their implications
- To advise on key dengue diagnostics targets and their interpretations
- Dengue diagnostic markers– What, When and Why/Where

INTRODUCTION

DENGUE VIRUS AND SEROTYPE TRENDS IN MALAYSIA



Dengue Serotypes in Malaysia (1990–2014)



Findings:

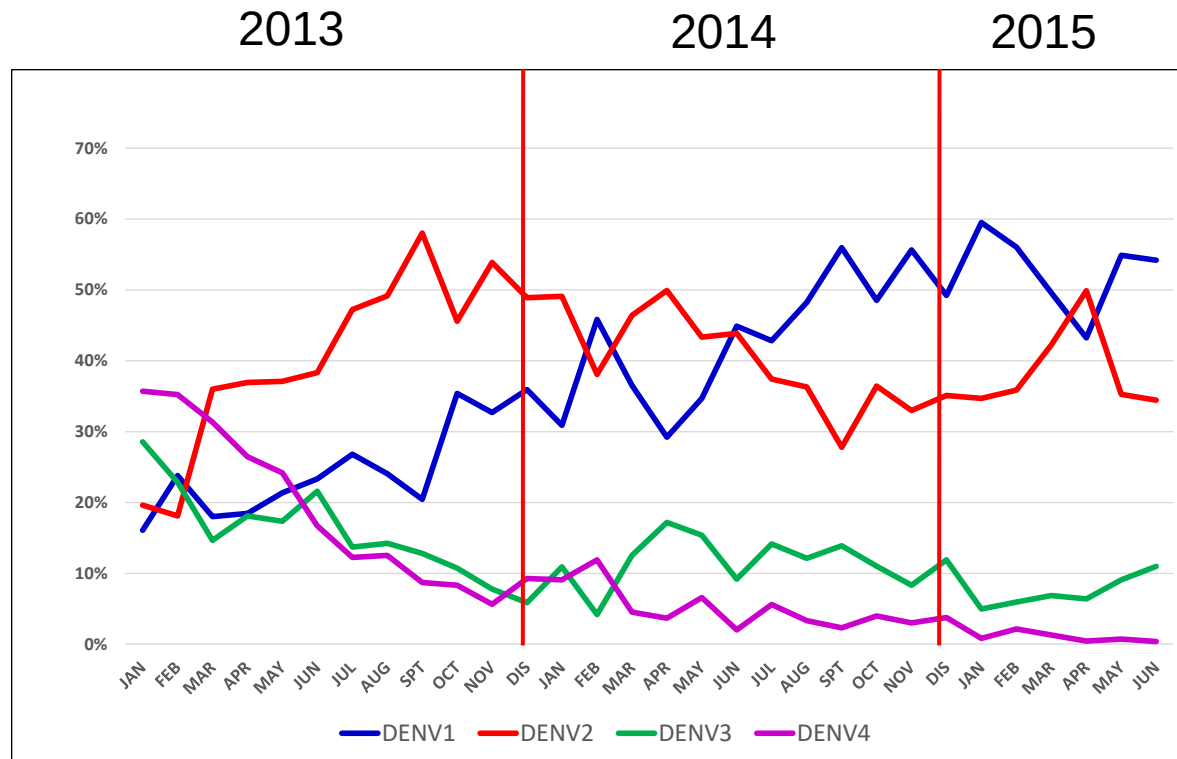
- (1) Den 1 and Den 2 pre dominate for the past 13 years
- (2) Den 3 pre dominate in early 1990s
- (3) Den 4 –background

INTRODUCTION

DENGUE VIRUS AND SEROTYPE TRENDS IN MALAYSIA



Dengue Serotypes in Year 2013, 2014 and 2015 (Jan–June)



Findings:

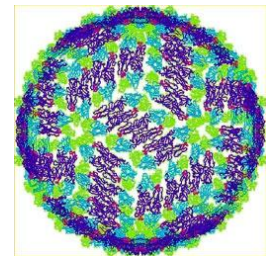
Den 1 and Den 2
co-pre dominate for
the past 2 years

Implications

DENGUE VIRUS AND SEROTYPE TRENDS IN MALAYSIA

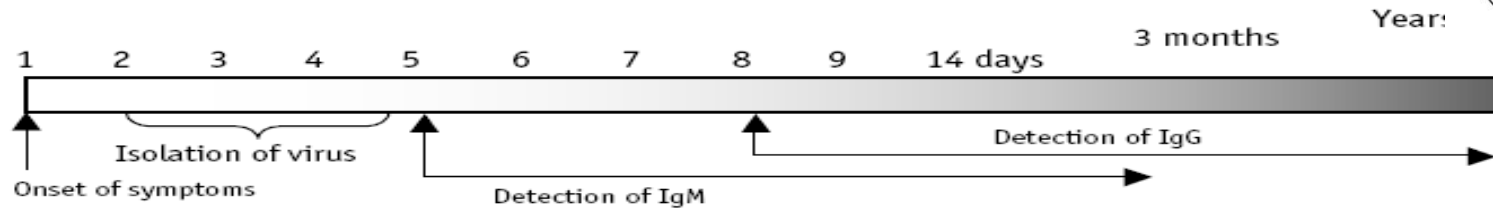


- The phenomenon of “double whammy” as both dengue 1 and dengue 2 co-predominate:- rather unusual as usually as a single serotype pre-dominates
- Increases risk of secondary dengue infections
- Long dengue epidemic- can result in the presence of a “fitter” virus- able to evade immune response and stay “longer in the community”
- Den 3 (which used to pre dominate in the 1990s) has not been a major player since 2001.



Diagnostics

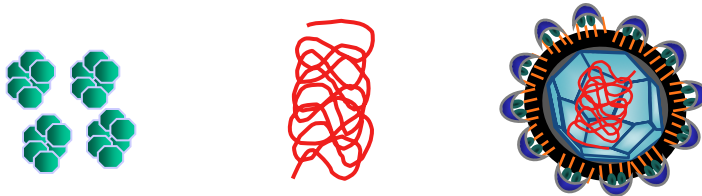
Diagnostic markers – What, When and Why



OPPORTUNITY

DIRECT METHODS

INDIRECT METHODS



**Antigen
Detection(NS1)**

**Genome
detection**

**Virus
Isolation**



**Serology
IgM**

**Serology
IgG**

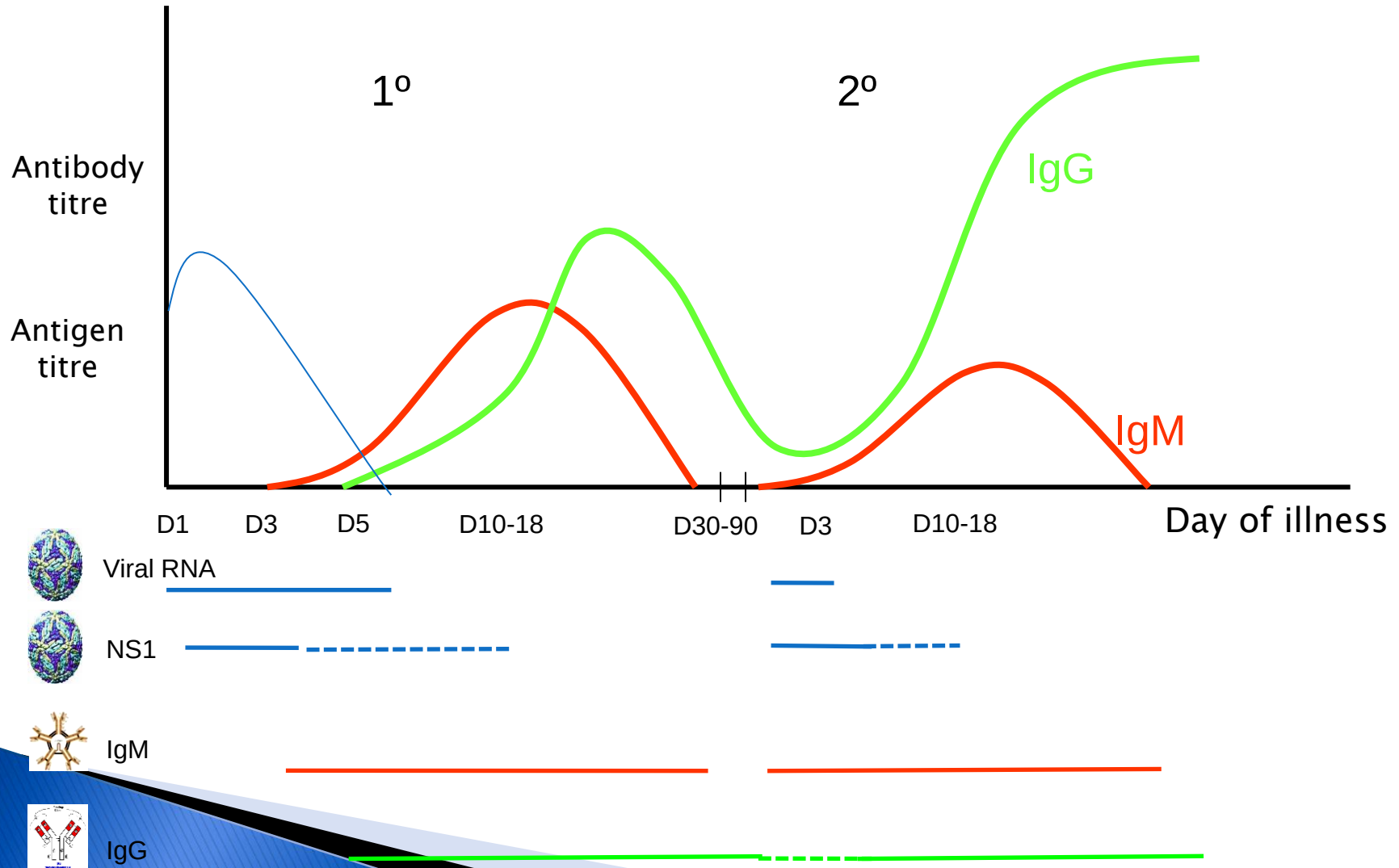
CONFIDENCE

Diagnostics

Diagnostic markers – What, When and Why



TIME COURSE OF DEVELOPMENT OF IMMUNE RESPONSE TO DENGUE



Diagnostics

Rapid Combo Test (RCT) for NS1 /IgM /IgG – What, When and Where



➤ **RCT** – can detect both – the presence of **virus** (during viraemia) and **antibodies** (during seroconversion)

➤ **Virus**– NS1 and **Antibodies**– IgM and IgG

➤ Hence the test has a longer detection window, thus reducing possibility of false negative

➤ The sensitivity and specificity are 93.9% and 92% respectively. (**Caution:**– The test must be read within the specified time~15–20 mins, if not may result in false positive)

➤ Available in Health Clinics, Emergency Dept, Some Labs



Diagnostics

Dengue NS1 ELISA- What, When and Where



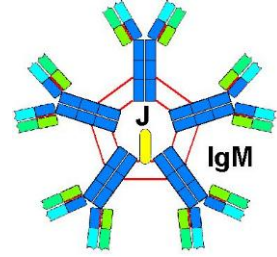
- **NS1** – The most important dengue virus protein for diagnostics



- Secretions of this protein is hallmark of flavivirus infecting mammalian cells (including dendritic cells, peripheral blood mononuclear cells etc.)
- **NS1** has been associated as prognostic marker for **severe dengue** among **prolonged viraemia cases (D5/D6)**.
- **Detection rate is better** in acute sera of **primary dengue (75–97%)** vs secondary dengue (60–70%)
- The sensitivity drops after day 5 of illness and becomes undetectable in convalescence phase
- Available in major microbiology labs in hospitals

Diagnostics

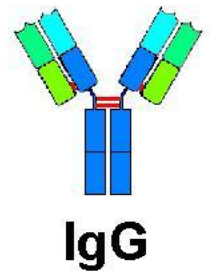
Dengue IgM ELISA– What, When and Where



- **IgM Capture ELISA** – most widely used serological test. Titre higher in primary vs secondary dengue
- **Primary**– IgM can be detected after **day 5** of illness in 80% cases and almost **93 to 99%** by day 6 to day 10.
- If negative IgM – should **repeat serum** after day 5 of illness
- **Secondary**– IgM can be detected after **day 7** (70%)
(Caution:– IgM titre usually lower as “blocked” by high levels of IgG. **28% of all secondary dengue missed if only IgM used**).
- Available in major microbiology Labs in Hospitals

Diagnostics

Dengue IgG ELISA– What, When and Where



- In **Primary and Secondary** dengue infections– IgG is detected in **100%** of patients by **Day 7** of illness.
- **IgG** – Recommended if IgM is still negative after Day 7
- **IgG titre** – useful to determine **secondary** dengue infections. Commercially available ELISA kits– incorporate threshold levels of IgG $\geq 1:2560$ titre – confirmed secondary dengue
- Available – Major microbiology labs in Hospitals

Recommendation 2

- Dengue rapid combo test or non-structural protein 1 antigen (NS1 Ag) should be taken as soon as dengue infection is suspected.
- If dengue IgM is negative before day seven, a repeat sample must be taken in recovery phase.
- If dengue IgM is still negative after day seven, dengue IgG should be done for diagnostic confirmation of secondary dengue infection.

Diagnostics

Dengue Viral RNA Detection by rRT-PCR– What, When and Where

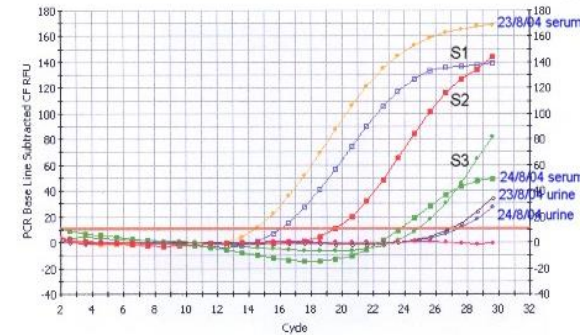


➤ Dengue Viral RNA Detection– Detection of dengue viral nucleic acid by Multiplex Real Time Reverse Transcriptase Polymerase Chain Reaction (rRT–PCR)

➤ Useful in early onset (during viraemia), up to Day 5/6 of illness

➤ **Able to detect and serotype simultaneously**– useful for surveillance as information about circulating dengue serotype useful as a predictor for dengue outbreak. (Surveillance for severe dengue in ICU/HDW)

➤ Also useful as one of the diagnostic tool in fatal dengue cases (samples include serum, liver biopsy and CSF)



Diagnostics

Dengue Viral RNA Detection by rRT-PCR– What, When and Where



- Sensitivity of rRT-PCR is hindered by Rnase enzyme and antibodies, which are also present in patients.
- Sensitivity best (**first 5/6 days**) vs NS1 antigen (**Up to first 9 days**) but highly specific due to use of probe.
- Test is costly (**~RM60 per test**), requires special temperature storage and fast transportation
- Available only in Virology Unit, IMR, National Public Health Laboratory, Public Health Laboratories & UMMC

Diagnostics

Dengue Viral Isolation– What, When and Where



- Dengue virus isolation– “Gold Standard” as the presence of virus is indicative of infection
- Useful only during “viraemic phase” and can take up to 21 days for results.
- Successful virus isolates can be used to perform full genome dengue virus sequencing– to identify dengue genotypes or mutations associated with viral virulence.
- Test is available at Virology Unit, IMR, National Public Health Laboratory & UMMC



Investigation of Post Mortem cases

- Suitable samples (ie. Liver biopsies and/or CSF– in encephalitis cases and serum) should be send to Virology Unit (Institute for Medical Research) or National Public Health Laboratory (NPHL,Sg Buloh) for further laboratory investigations.
- Serum – tested for NS1 or IgM or IgG as well (Hospital)
- Tests – rRT–PCR and virus isolation

Diagnostics

Diagnostic approaches for Post Mortem Cases– What, When and Where



Recommendation 3

- A repeat dengue serology should be obtained at the time of dengue death.
- Suitable specimens for viral isolation and/or real time reverse transcriptase polymerase chain reaction (RT-PCR) and/or non-structural protein 1 (NS1) antigen detection should be done for confirmation of dengue infection.



- Rapid Combo Test– very useful method as detection of NS1 (virus) and IgM/IgG (antibodies) can minimize false negative results
- Dengue NS1 antigen is also useful as early dengue marker and has potentials to be used as severe dengue prognostic marker.
- Onset of Disease– NS1 (till D9), dengue rRT–PCR (till day 5/6)
- Dengue IgM (up to 2 months) and IgG (determination of titre)
- Dengue Deaths– liver biopsy/ CSF/ Serum– Dengue rRT–PCR

TAKE HOME MESSAGE



Clinical History	Test	Result	Interpretation
History of fever less than 5 days	Dengue NS1 Ag or RCT	Positive	Acute dengue infection.
		Negative	Dengue infection still cannot rule out. Suggest to send second sample for Dengue IgM after day 5 of fever
History of fever more than 5 days	Dengue IgM	Positive	Presence of detectable IgM antibody. Suggestive of recent dengue infection
		Indeterminate	Advice to repeat the test.
		Negative	The result does not rule out dengue infection. Advice to send repeat sample for dengue IgM after day 7 of fever or ask for Dengue IgG test.
History of fever more than 5 days and Dengue IgM and /or NS1 was negative	Dengue IgG	Positive	Elevated IgG levels are seen in acute or past infections. A titre of equal or more than 1:2560 is consistent with acute secondary infection.
		Indeterminate	Advice to repeat the test if clinically indicated.
		Negative	No detectable elevated IgG antibody. The absence of elevated IgG is presumptive evidence that the patient does not have secondary dengue infection.

THANK YOU