Dengue Summit 2008

An endemic disease affecting tropical and sub-tropical countries, dengue is considered a major health problem by the WHO. It has been estimated that 2,500 million people are at risk worldwide with 50-100million cases annually resulting in 25,000 deaths. Early diagnosis is crucial to enable prompt vector control and transmission reduction, with the aim of attaining lower morbidity and mortality rates. Key health officials and dengue experts from the region shared updates at a recent summit organized by Malaysian Society of Parasitology and Tropical Medicine and sponsored by Bio-Rad Laboratories held in Kuala Lumpur.

Dengue on the Rise – Why?

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Dengue occurrence has been steadily increasing in Malaysia. The increase can be attributed to a number of factors such as climate changes, vector control efficacy and a culture dependent on disposable crockery, which increased breeding sites when improperly disposed of.

Dengue vectors include species such as *Aedes aegypti*, *Aedes albopictus* and *Aedes niveus*, with the latter infecting monkeys. An infected mosquito takes an average of 7 days to become infective and remains infective for life up to a week.

Intermittent or low rainfall encourages *Aedes* mosquitoes to breed while rain followed by high temperatures adds to the risk.

Chemical control is a way to interrupt the transmission. However, sub-lethal dosages of insecticide cannot alter the vectoral capacity of the *Aedes* mosquito. Other reasons for control failure include inappropriate timing of application, choice of equipment or insecticide, dosages, vehicle speeds and droplets profile.

Prevention is important to combat dengue, relying on new methods of diagnosis to detect infection and control the spread. Advances in dengue research have emerged, such as rapid detection of dengue virus in vectors and NS1 Ag tests, making it possible to prevent more cases with earlier diagnosis.

The Importance of Dengue Early Diagnosis

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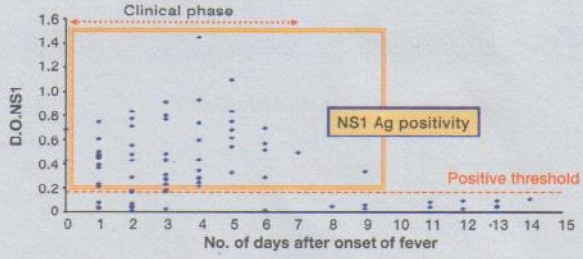
No vaccines are currently available for dengue, making prevention and early diagnosis crucial. The dengue virus codes for 3 structural proteins and 7 non-structural (NS) proteins. It is shown that NS1 proteins circulate in the sera of patients on the first day onset of fever, giving a high percentage of positive results on average in diagnostic tests.[1] (**Figure 1**)

There are currently 3 types of Dengue NS1 Antigen (Ag) tests (**Table 1**). Dengue NS1 Ag test has an overall sensitivity of 88.7%, compared to reverse transcriptase-polymerase chain reaction (RT-PCR) (85.0%) and viral culture (94.1%), with no significant difference among the 4 dengue virus serotypes.[2]

Figure 1. NS1 Protein circulates in the sera of patients during the clinical phase



Alcon *et al.*, *J Clin Microbiol*, 2002

The use of NS1-based diagnostics is encouraged for the first few days of dengue as levels of IgM have yet to be detectable. An evaluation of various diagnostic methods showed overall higher sensitivity for Dengue NS1 Ag, especially in primary dengue.[3] (**Table 2**) A prospective study also supported the use of NS1 Ag in early diagnosis after fever onset, with IgM determination increasing the diagnostic rate after day 4.[4]

Table 1. Comparison of Various Dengue NS1 Ag Tests

Test	Remarks
Pan-E Dengue Early ELISA	- Short assay time - Ready-to-use colour coded reagents
Dengue NS1 Ag STRIP	- Individual testing - Lateral flow immuno-chromatography technology for qualitative detection - Used on human serum or plasma - 15 minutes to results
Platelia™ Dengue NS1 Ag	- One step format assay - Simultaneous distribution of Diluent, Sample and Conjugate - Incubation at 37±1°C

Table 2. Laboratory Diagnosis of Acute Dengue Virus Infection

Methods	Primary Dengue (N=184)	Secondary Dengue (N=29)	Total (N=213)
Platelia Dengue NS1	N=179 (97.3%)	N=20 (70.0%)	N=199 (93.4%)
Viral culture	N=136 (73.9%)	N=9 (31.0%)	N=145 (68.1%)
RT PCR	N=120 (65.2%)	N=22 (75.9%)	N=142 (66.7%)
IgM	N=60 (32.6%)	N=17 (58.6%)	N=77 (36.2%)

Serological diagnosis includes ELISA, which can be used for IgG or IgM detection, and haemagglutination inhibition (HI) assays. Between days 0 to 4, MAC-ELISA is unable to detect dengue accurately, in contrast to the more effective Dengue NS1 Ag. Therefore, a diagnostic strategy combining Dengue NS1 Ag testing of serum collected within 5 days of the onset of fever and MAC-ELISA for serum samples collected in the early convalescent phase would potentially make it possible to diagnose at a sensitivity of 91.7%.

References

1. Alcon S, et al. *J Clin Microbiol* 2002;40(2):376-81.
2. Dussart P, et al. *Clin Vaccine Immunol* 2006;13(11):1185-9.
3. Kumarasamy V,et al, *J. Virol. Methods* 2007;140(1-2):75-9.
4. Chuansumrit A, et al. *Ped. Infect. Dis. J.* 2008;27(1):43-8.

The Right Tool at the Right Time

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The importance of using the right test at each stage of the disease was emphasized. The profile of IgM, IgG, IgA and the virus are present at different stages of the disease, making them possible markers of the disease. Serology testing, which assays for IgM, IgG, can be used at specific windows. (**Table 3**, **Figure 2**)

During the first few days of viraemic or fever phase, detection of NS1 is prominent for the first 6 days, while RT-PCR has higher sensitivity. IgM/Capture IgG tests have significantly low sensitivity for the first few days, overtaking the other forms of testing by the first week. (**Figure 3**)

It is cost-effective to screen for dengue using real-time RT-PCR followed by rapid single tube multiplex

Figure 2.

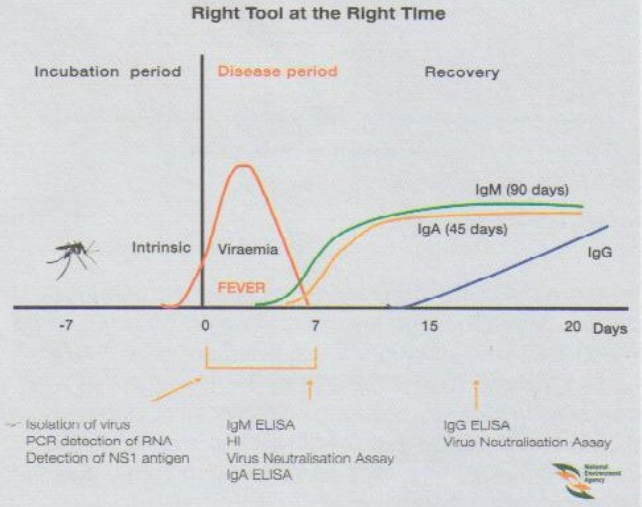

Figure 3. Detection of Viral Antigen, NS1 in Viraemic/Fever Phase

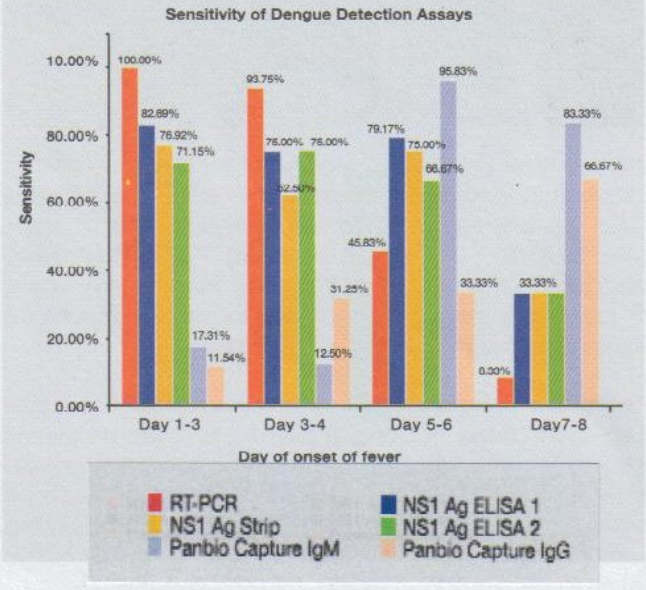

Table 3. Serology Testing at Environmental Health Institute

Days after onset of clinical symptom	Day 7		Day 7 -15	Day 15-20	
Tools	IgM Assays		Neutralisation Assays	IgG Assays	
	ELISA	Duo Cassette		Indirect IgG	Capture IgG
Advantages	- Reliable at the right time - >90% PCR positive cases subsequently sero-convert	- Immediate results	- Useful for sero-epidemiological study, especially in absence of IgM	- For indirect IgG with low levels due to previous or primary infection - Useful for sero-epidemiological study	- For secondary infections - Useful for sero-epidemiological study
Disadvantages	- Analysis by batch causes delay - SLE patients have false positive	- Less sensitive than ELISA - False positives - Misdiagnosis of dengue as chikungunya	- Laborious - Time consuming - Unreliable in >2 serotypes	- Paired samples required - Non specific, cross reacts with other flaviviruses	- Paired samples required

RT-PCR for serotyping. From the fifth day, IgM can be used as a reliable marker of dengue. NS1 antigen marker sometimes fills the gap of 5-6 days (from fever onset) when both IgM and PCR may be negative.

Misdiagnosis is a possibility. Chikungunya is transmitted by the same vector as dengue. IgM produced in response to it may last up to a year. Rheumatoid factors produced during other diseases can cause false results when testing for dengue or chikungunya. NS1 and PCR testing provide more definitive results when testing for dengue.

Early detection is important for Singapore's dengue control program, with an integrated information system, effective public education and law enforcement to combat dengue in susceptible areas.

Dengue-infected Patient Management

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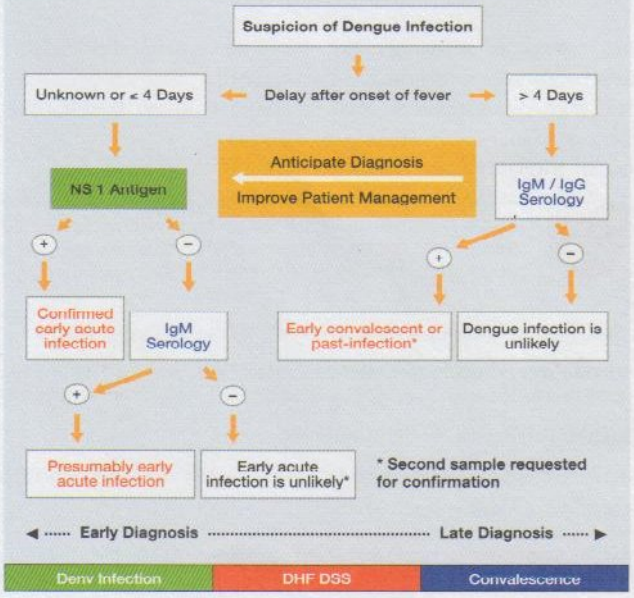
Diagnosis of dengue involves virus isolation, genome detection, antigen detection and serological testing. Since March 2006, a syndromic surveillance system with a network of doctors and weekly reports of cases has been set up in French Guiana. Most laboratories now use Dengue NS1 tests.

Symptoms of dengue include fever >38.5°C, cephagia, retro-orbital pain, myalgia, arthralgia, lumbalgia and thrombocytopenia. A small number of severe cases may involve haemorrhagic fever with or without shock syndrome, rhabdomyolysis, exhaustion syndrome, and encephalitis-like syndrome.

Dengue is confirmed according to the results of the NS1 test. Serum samples are collected between day 0 and 4 after fever onset. If NS1 results are positive, there is strong evidence, as specificity is 100%. If NS1 is negative, results are sent for RT-PCR to rule out dengue. MAC-ELISA serology is only recommended for serum samples collected after day 5. (**Figure 4**)

Figure 4. Summary of Dengue Testing Protocol



Round table discussion by countries

Dengue Situation in Indonesia

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Spread by the main vector Aedes aegypti, dengue requires clinical confirmation to differentiate it from rubella, measles, arborovirosis, leptospirosis or avian flu. It can be diagnosed by viral isolation, serology, antigen detection or genome detection.

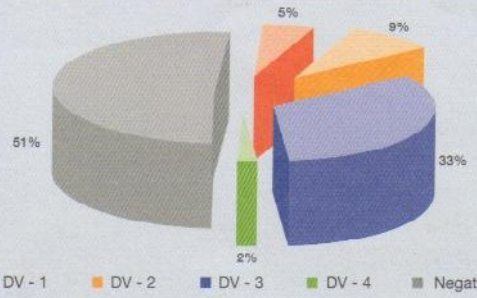
Based on an RT-PCR test on 600 samples of suspected cases, more than half were serotypically classified as DV-3, with only 2% as DV-4. Diagnosis can be confirmed using kits testing for antibody or antigen and PCR. (**Figure 5**)

Main Points of Indonesia's National Programme

- Epidemiology surveillance
- Eradication of vector and outbreak control
- Clinical management
- Partnership
- Community participation
- Training
- Research

Outbreak response involves an emergency team and prompt communication, with surveillance and a health referral system. A partnership between public and private sector can be forged, enabling social mobilization.

Figure 5. DHF's samples distribution based on RT-PCR test result



From 600 samples (Susp Dengue Fever), the RT - PCR 90 positive result were: DV-1 = 9, DV-2 = 18, DV-3 = 65, DV-4 = 4

A national network is in place to ensure proper reporting procedures between public health officials, scientists and clinicians, involving personnel such as pathologists and epidemiologists. Sample information is obtained and analyzed, after which results are shared. This encourages optimal surveillance and control, with the National Institute of Health Research and Development coordinating communication material between universities, hospitals, National Board for Technological Screening and Assessment, WHO and NGOs.

Dengue in Malaysia

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A public health problem, dengue has an increasing mortality rate among adults in Malaysia, with DHF causing more deaths compared to DF. 1/3 of dengue cases in the country are serologically positive, with the states of Kuala Lumpur and Selangor showing higher rates of incidences.

Dengue policies in Malaysia are supported by laws such as the Malaysia National Health Policy 2007, which aims to reduce incidence and mortality rates of communicable diseases such as dengue, one of the notifiable diseases in the country. The Destruction of Disease-bearing Insects Act also strives to reduce vector sources, making it an offence to breed mosquitoes.

Key components of dengue control strategies include early diagnosis and treatment, prompt notification, prompt vector control response, proper case management, health education and community mobilization. This requires intersectoral collaboration and surveillance systems with the use of e-notices and programmes to obtain epidemiological data and monitor vector control activities.

Dengue Surveillance and Control Initiatives

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Current measures to control the spread of dengue such as vector eradication that depend on laboratory diagnosis have been found to be ineffective in an outbreak. A more effective strategy would require early rapid detection and serotyping of dengue viruses. Active surveillance and monitoring are required even before an outbreak. This can be an orchestrated effort, with contribution from various sectors, ranging from building contractors, architects, NGOs, environmentalists and pest control professionals.

In laboratories where diagnosis is done solely using in-

house IgM capture ELISA, there is a high possibility that early infection will not be detected. In some hospitals, negative patient sera will be put into cell culture for virus isolation. 80% of samples will be negative after at least two passages, therefore wasting resources. An efficient method is to compliment IgM capture ELISA with other early detection methods such as NS1 ELISA and PCR, together with a program where positive patients are counseled to prevent the spread of dengue. This includes voluntary isolation and the use of mosquito nets.

Post-outbreak, a thorough assessment of the outbreak and improvement of monitoring and surveillance systems can be undertaken to prevent future outbreaks.

Continuous research needs to focus on predisposing factors of DHF, discovering novel antivirals and drugs which prevent vascular leakage, improved diagnostics and understanding dengue virus phylodynamics. There should be good reference reagents to assess current diagnostics.

Dengue Case Management

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Dengue morbidity and mortality can be reduced through a series of actions such as surveillance, prevention, case management, research and outbreak response.

A weekly dengue update is generated, with information obtained from all 17 regions of the country. However, problems encountered with the findings include different case definitions, case reporting and an absence of laboratory surveillance in other regions.

Dengue research has been harmonized at the national level, encompassing topics such as diagnostics, vaccine production and communication. Vector control has been integrated with the health services under sanitary inspectors, at provincial, regional and national levels.

Social mobilization is crucial to support this movement. Taking the message to the people involves printed material and campaigns such as 4S (**Table 4**) and the 4 o'clock habit, which refers to households turning water containers upside-down in the afternoon.

Table 4. 4S Actions

1. Search and destroy breeding places
2. Self Protection – Reduce exposed skin and use repellent
3. Seek early medical advice
4. Say 'no' to indiscriminate fogging

Singapore's Dengue Control Programme in the Face of New Challenges

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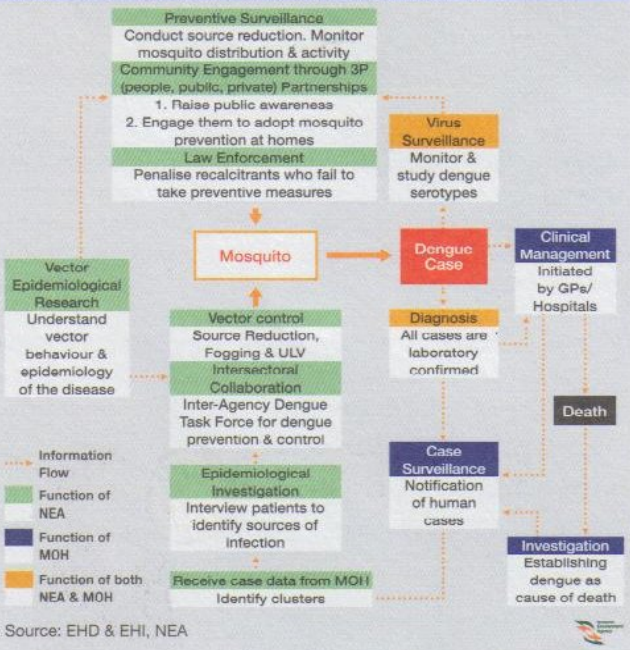
Dengue is a notifiable disease in Singapore. An organised system linking the National Environment Agency and the Ministry of Health in Singapore ensures information flow. This integrated dengue control strategy (**Figure 6**) involves field and laboratory surveillance, inclusive of virus, vector and patient factors.

Intensive source reduction requires eradicating existing breeding sites and identification of potential sites. Under the Control of Vectors and Pesticides Act, occupants found with breeding sites will be penalized, and the pest control industry professionalized.

Audit checks on property are conducted, with daily surveillance, Geographical Information System Clustering and Analysis of sites and cases. This includes Aedes breeding, serotype distribution, case distribution, active clusters and sensitive areas.

Figure 6. Coordination with Health and Environment



Early cost-effective diagnostics such as NS1 Ag tests, which allow prompt activation of dengue control, are offered to private clinics. In event of an outbreak, surveillance should be enhanced, with search efforts and community engagement intensified. Indoor misting is conducted routinely, replaced with outdoor fogging when an adult Aedes population is detected.

Patient samples are also used for virus surveillance. Serotype switch should be used as a warning sign. A localized emergence of uncommon serotypes should be monitored.

Higher temperatures result in an increase in dengue cases due to faster emergence of adult mosquitoes, increased biting rates and increased viral replication rates. Research is underway in disease epidemiology, vector research, diagnostics, surveillance and surveys.

Dengue / Dengue Haemorrhagic Fever in Thailand

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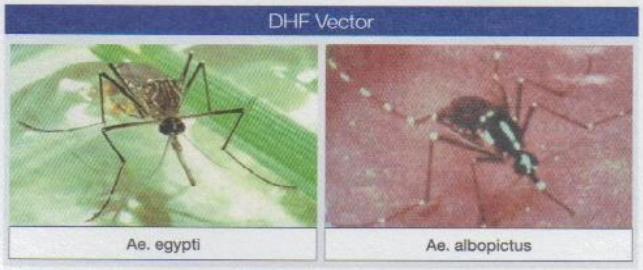

The peak of reported dengue cases coincides with the rainy season, occurring mostly in the central region. Serological studies show a change in dengue serotypes each year in endemic areas.

Dengue fever is a notifiable disease that is monitored by the Bureau of Epidemiology, with cases passing through the district health office and provincial health office.

Serological testing determined the most prevalent dengue serotypes as DEN-1 and DEN-3. Larvae control and evaluation were also conducted, with 60% of larvae discovered indoors and 40% outdoors.

Risk factors affecting dengue morbidity in Thailand include the increase in man-made containers, seasonal variation, lack of community participation and public information, unsustainable source reduction and lack of concern of adult groups by clinicians.

Mortality is affected by lack of early diagnosis, inadequately trained healthcare workers, late medical consultation, self-treatment, poor healthcare management during epidemics and a poor reference system among certain hospitals.

The National Dengue Prevention and Control Plan's Strategies.

1. Empowering individuals and communities
2. Environmental modification to control breeding sites
3. Health promotion
4. Multisectoral networking
5. Proper administration and management
6. Technological development of prevention, control and treatment of Dengue Fever

These plans have proven significant, with the development of technical material, dengue control campaigns and education of both laymen and clinicians. International conferences have also been organized.

Current research focuses on developing vaccines and investigating the effectiveness of source reduction, adulticide and larvae control. A rapid response to epidemics and established control in the provinces can be expected.

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