

1 **Review Paper**

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3 **A Review of Dengue Fever with Special Reference to Malaysia**

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6

7 **Abstract**

8 **Introduction:**

9 Dengue is is the most prevalent viral mosquito-borne disease, with over 2.5 billion humans at risk given its
10 endemicity in more than 100 countries.

11 Globally, 50-100 million cases of dengue occur annually, with approximately 0.7% resulting in dengue
12 haemorrhagic fever (DHF), and 22,000 deaths.

13 In 2017, there were 83,849 reported cases of dengue fever in endemic under-reported Malaysia, with 177 deaths.

14 One of our authors (Verasingam K 2006) here, earlier published an article in the Med J Malaysia titled “Dengue
15 Fever in Malaysia: Time for Review?” This Article is meant as a sequel.

16 **Method:**

17 The Authors here narrate from their own personal-experiences, as well as from reviewing existing-literature.

18

19

20 **Results and Conclusion**

21 Clinical Management has brought about vast improvements in mortality and morbidity. Similarly, great
22 advancements in Laboratory Diagnostics. Prevention and Control methods have been desiring of greater
23 achievements, but also show greater promise with Newer Insecticides, Innovative Methods and Vaccines.
24 Dengue Fever would very likely become near-eradicated just like all other vaccine-preventable diseases, once
25 comprehensive mass-vaccination programmes are available globally, using safe and very-effective tetravalent-
26 vaccines soon to be available.

27

28 **Key-words:** dengue fever; Severe dengue; epidemiology; clinical management; laboratory diagnosis; control
29 and prevention; dengue vaccine

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32 **Epidemiology**

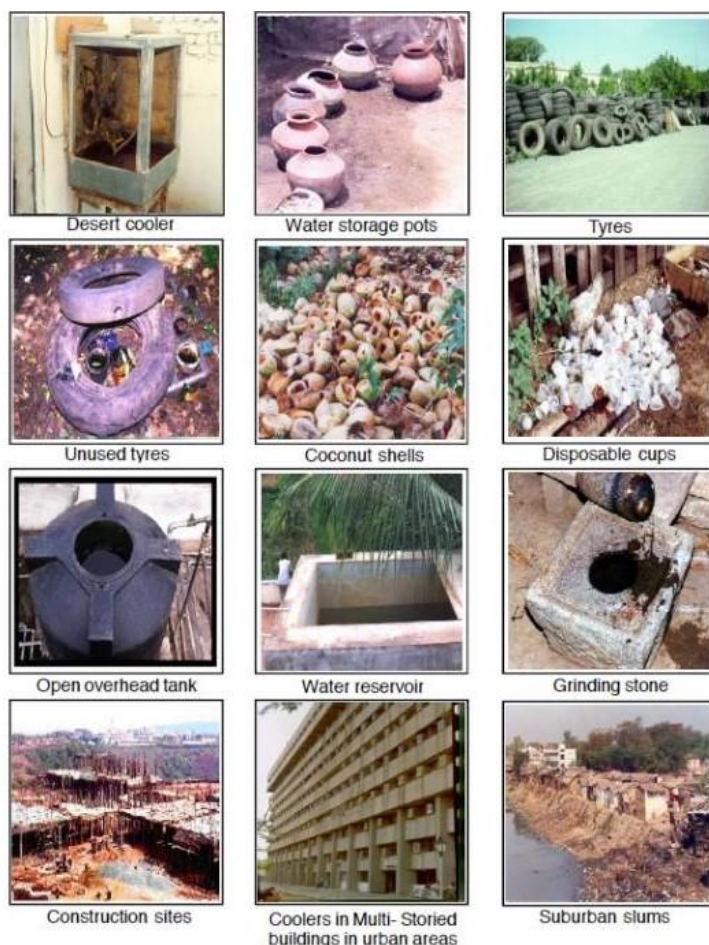
33 Dengue is considered the most important mosquito-transmitted viral disease in terms of morbidity and mortality.
34 It is the most prevalent viral mosquito-borne disease, with over 2.5 billion humans at risk of exposure given its
35 endemicity in more than 100 countries, compared to nine countries in 1970. 1 - 11

36 Dengue fever is a benign, acute febrile syndrome occurring mainly in tropical regions. In a small proportion of
37 cases, the virus causes increased vascular permeability that leads to a bleeding diathesis or disseminated
38 intravascular coagulation (DICC) known as dengue haemorrhagic fever (DHF).3

39 The term Severe dengue is used classically to describe DHF and DSS, but some authors include Dengue With
40 Complications (DWC) as Severe dengue. Such complications, outside of DHF and DSS, include mostly
41 neurological complications (which are commoner in children) and liver involvement.

The vectors are *Aedes aegypti*, which breeds in and around houses and buildings, and the smaller *Aedes albopictus* which breeds outdoors. They are day-biting mosquitoes, whose peak biting-hours are dawn, early morning and dusk. 1 – 11 Besides other factors, the bite of the *A. aegypti* is more likely to cause severe dengue (DHF and DSS). 12 – 14 Major sources of *Aedes*-breeding are at construction sites, solid-waste dumps, open-spaces and in factories. 1 – 11

Fig 1: Common *Aedes aegypti* breeding-sites, including in Malaysia



The WHO estimates that 50-100 million cases of dengue occur annually, with approximately 500,000 of those cases (0.7%) resulting in dengue haemorrhagic-fever (DHF), with an estimated 22,000 deaths per year, mostly in children. In 20-30% of DHF cases, the patient develops shock, known as the dengue shock syndrome (DSS). 1, 4, 5 – 8 Studies in the Philippines and Indonesia reveal that 70 – 80% of dengue infections are asymptomatic. 15 – 16

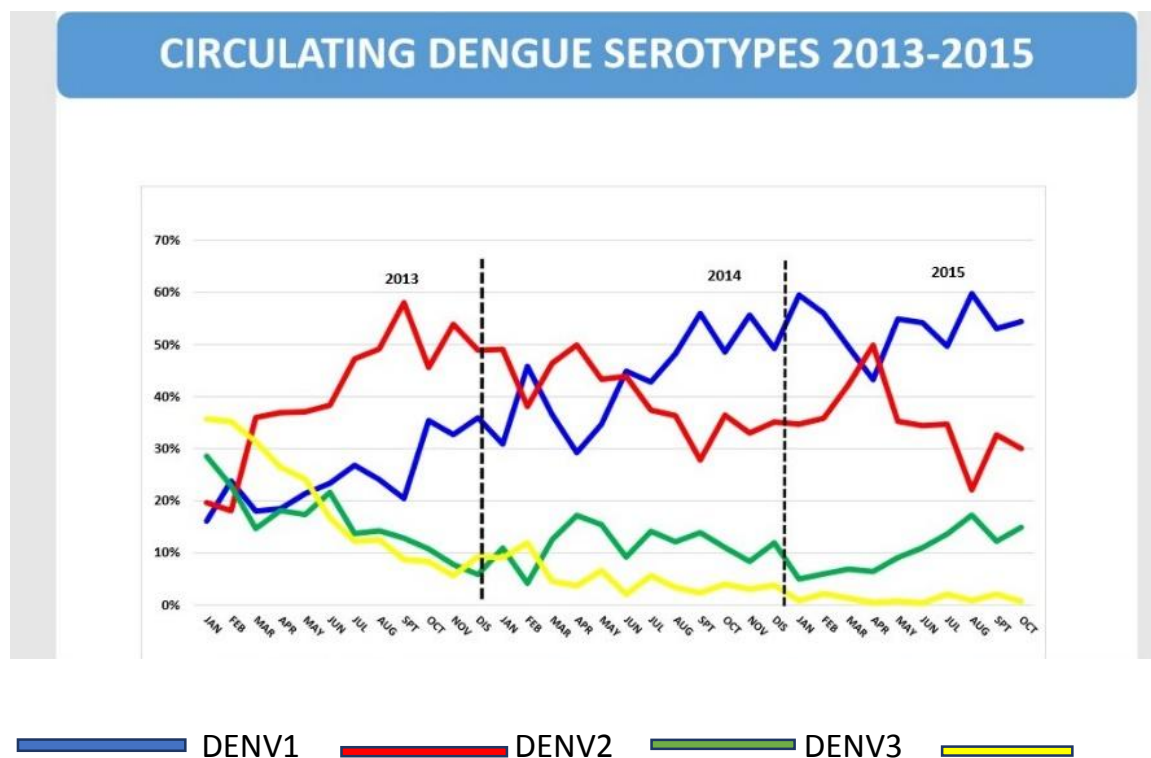
The term Severe dengue is used classically to describe DHF and DSS, but some authors include Dengue With Complications (DWC) as Severe dengue. Such complications, outside of DHF and DSS, include mostly neurological complications (which are commoner in children) and liver involvement.

There is no specific treatment for dengue/ severe dengue, although there is promise of effective anti-viral drugs at least to prevent mild dengue converting to severe dengue. 7 – 8 Early detection and access to proper medical care, however, lowers fatality rates below 1%. 1

There are 4 distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3 and DEN-4). Recovery from infection by one provides lifelong immunity against that specific serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes increase the risk of developing severe dengue. 1 – 11 In Malaysia, various serotypes steadily begin to predominate in the various years – the present predominant strain being DENV3 replacing DENV1 and

65 DENV2. 17 The prevalence of individual serotypes varies across different geographies, countries, regions,
66 seasons and over time. 1 – 11

67 **Fig 2: Circulating dengue-serotypes in Malaysia 2013 – 2015. 17**



68

69

70 — DENV1 — DENV2 — DENV3 —

71 DENV4

72 In Malaysia, there is a male-preponderance at 57%. The number of cases steadily increases from a moderate
73 among toddlers to a peak in the early twenties, before steadily falling to a moderate again in the late 40s, and
74 before tapering down to a low in the elderly - although the highest incidence is among the working and school-
75 going age groups. 17, 9 – 10

76 Severe dengue (DHF and DSS) may be commoner in females and those with co-morbidities such as diabetes-
77 mellitus and obesity. The case-fatality rate in Severe dengue appears much higher in females. (José de Jesus
78 Dias et al 2017) (Sing-Sin Sam et al 2013)

79 The disease is endemic in Malaysia since the 1980s. 3, 9 – 10, 18 - 19 Shepard DS et al (2012) say that because
80 Malaysia has a passive-surveillance system, the number of dengue cases is under-reported. 18

81 In Malaysia, a “dengue outbreak” is defined as two cases occurring in an area over 14 days, while a “dengue
82 hotspot” is when the outbreak is sustained more than 30 days. (Petaling Jaya City Council, ibid 2018).

83 Nur Azila MA et al (2011) in studying 1000 subjects aged 35-74 years old, found 91.6% to be dengue
84 seropositive. The sero-prevalence increased with every 10-year increase in age. Gender and ethnicity were not
85 factors. There was no difference between urban and rural areas. 20

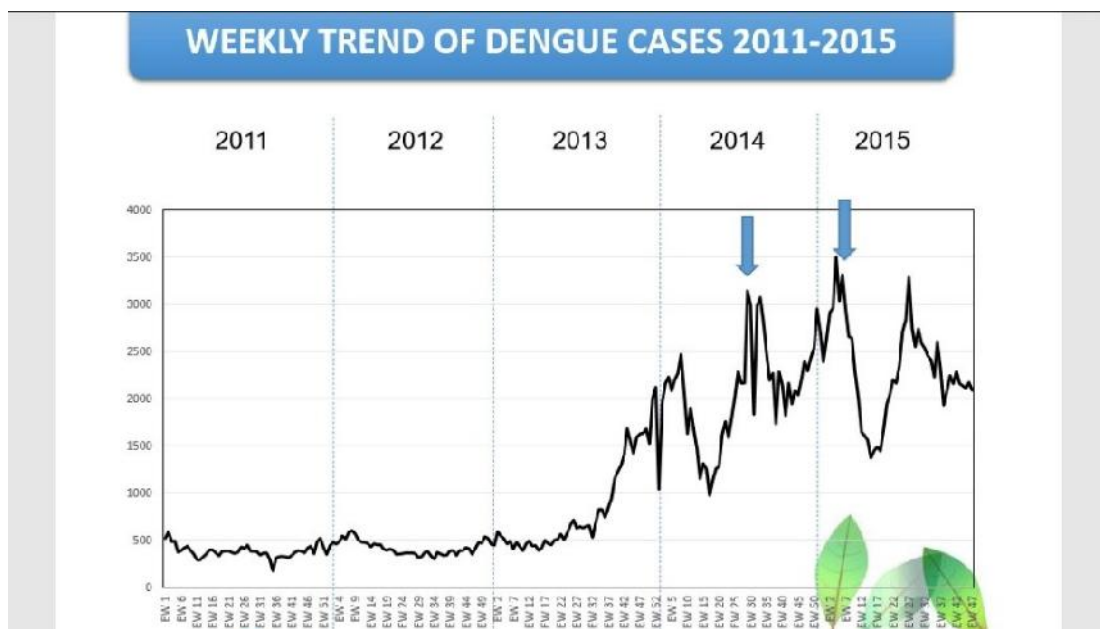
86 In 2017, there were 83,849 reported cases of dengue fever nationwide with 177 deaths, which is a conspicuous
87 reduction from the immediate previous years. 21 - 22

88 The Health Ministry attributes its recent achievements to the integrated efforts of various ministries, agencies,
89 society and individuals, although this reduction may be a part of the six-year pattern in the country discussed
90 below. 19, 21 - 22

The National Dengue Task Force (NDF), which comprises seven ministries and various agencies, was set up since July 2014 to mobilize agencies and members of public in dengue prevention and control activities. 21 – 22 Besides the NDF, there is the National Dengue Committee. 17

It was after 2013, that a sharp increase in the incidence was noted in Malaysia, which has remained sustained. 17 The contributing factors for this sharp increase are thought to be serotype-shift, mobility of the population, climate change, human behaviour, poor environmental sanitation and ineffectiveness of vector-control activities. 3, 17

Fig 3: Weekly-trend of Dengue-cases in Malaysia, 2011 – 2015. 17



In the Malaysian context, health reforms in the late nineties that integrated the vertical organizational structure of the Vector Borne Disease Control Programme with the general health services resulted in loss of technical expertise as well as constraints in funding, as limited health resources were moved to other competing programmes under the Ministry of Health which is thought to be the cause of the sharp-increase, coupled with local-governments being made responsible in some instances. After years of neglect, cities like Greater Kuala Lumpur, Penang, Johore Bahru, Seremban and Melaka have become hyper-endemic for dengue transmission, where more than one virus serotype are circulating. 11

While Wiwanitkit V (2010) says that the application of vector-control methods is labour-intensive, requires discipline and diligence, and hard to sustain. 23

In Malaysia, outbreaks of dengue tend to recur in six-year cycles, consisting of four years with high numbers of cases followed by two years with relatively low-numbers. However, the annual average incidence of reported dengue cases in successive six-year cycles has been increasing. 19

Shepard et al (2012) estimated an economic burden of dengue illness in Malaysia at US\$56 million per year, which is approximately US\$2.03 per capita. They say that the overall economic burden of dengue would be even higher if they had included costs associated with dengue prevention and control, dengue surveillance, and long-term sequelae of dengue. 18

Packierisamy PR et al (2015) estimated the costs of dengue prevention. In 2010, Malaysia spent US\$73.5 million, or 0.03% of the country's GDP, on its National Dengue Vector Control Program. If innovative technologies for dengue vector control prove efficacious, and a dengue vaccine was introduced, they say substantial existing spending could be rechanneled to fund them. 19

Clinical Management of dengue

121 The management of dengue fever is mainly supportive, with adequate fluid replacement and antipyretics. So far
 122 there is no licensed therapeutic-drug available for the treatment of dengue, although active-research is ongoing.
 123 24 - 25 Fever should be treated with paracetamol. Non-steroidal anti-inflammatory drugs (NSAIDs) should not
 124 be used as they are associated with bleeding risks and Reye's syndrome. Most patients can be managed as
 125 outpatients, with regular monitoring. It is recommended that patients be provided with a monitoring-chart. 26

126 The patient's hemodynamic status (blood pressure, pulse rate and volume) and full blood count (haemoglobin,
 127 haematocrit, white blood cell count, platelets count) require monitoring on a daily basis. Patients require
 128 education about warning signs and need to be advised to seek urgent medical-attention in case of warning signs.
 129 Severe abdominal-pain, recurrent vomiting and/or diarrhoea, inability to tolerate orally, lethargy, irritability and
 130 altered consciousness, cold peripheries and shortness of breath are warning signs associated with Severe dengue
 131 outcome. 27 - 30

132 Dengue fever has three phases: febrile, critical (plasma leakage) and convalescent (re-absorptive) phases. 8

133 Leptospirosis, typhus, malaria, typhoid and other viral exanthemas (especially measles, due to air-borne
 134 transmission) need to be excluded as a cause of fever. Chikungunya virus causes similar symptoms and signs
 135 and are transmitted by the same mosquito vector, *Aedes aegypti*. In studies comparing these two diseases, joint-
 136 pain was reported somewhat more often in chikungunya, whereas abdominal pain and leukopenia were more
 137 common in dengue. 32 - 34 The presence of typical skin rash, 'isles of white in a sea of red', thrombocytopenia,
 138 and leucopaenia supports a diagnosis of dengue.

139 The febrile-phase occurs 4-7 days after being bitten by an infected-mosquito and it lasts between 2 and 7 days.
 140 In this phase, there is high-fever (described as saddle-back fever), headache, retro-orbital pain, myalgia, typical
 141 macular-rash and facial-flushing. 35 - 36 There may be petechiae and minor gum bleeding, leucopaenia and
 142 thrombocytopenia. After about 3 to 5 days of fever, the patient enters into the critical-phase.

143 Critical-phase occurs when the temperature settles (defer-vescence) typically during day 3 to 7 of dengue. 27, 35
 144 - 37 Plasma leakage may occur during this period. In a patient who is not improving clinically despite the defer-
 145 vescence, besides a rising haematocrit and the presence of pleural effusion and ascites, it suggests plasma-
 146 leakage. Plasma-leakage must be detected early and treated to prevent it from progressing to severe
 147 decompensated and intractable shock - ultimately leading to multi-organ failure and death. Patients with
 148 coexisting conditions such as diabetes mellitus, pregnancy, obesity, renal-failure, advanced age and chronic
 149 haemolytic-anaemia have a higher risk of dengue complications. These group of patients may need hospital
 150 admission for close-observation.

151 Intravenous 0.9% saline (normal saline) has been widely used as fluid-replacement. Isotonic-solutions such as
 152 0.9% saline, Ringer's lactate or Hartman's solution is preferable for fluid-replacement. Intravenous fluid-
 153 replacement should not be for more than 24-48 hours, as the immunological-process causing plasma-leakage
 154 usually settles within that time-frame. Judicious fluid-administration must be practiced to maintain a good
 155 perfusion and normal vital signs, besides a minimum urine-output of at least 0.5 ml/kg/hr. Haematocrit must be
 156 particularly monitored. In the absence of a baseline haematocrit (hct), the median values of a normal
 157 haematocrit among Malaysian population is used i.e. for males 60 years and below - hct 46%, males above 60
 158 years - hct 42%, while for all females - hct 40%. 38 Additional laboratory investigations, including
 159 arterial/venous blood gas, serum lactate and bicarbonate improves the accuracy of fluid-replacement.

160 Studies have shown that there is no difference in overall-outcome and mortality between use of crystalloids
 161 versus colloids. 39 - 41 Colloids appear to be more useful as initial-resuscitation in patients with intractable-
 162 shock as it restores cardiac-index and haematocrit faster than crystalloids. Fluid-infusions must be closely
 163 monitored and adjusted regularly. Fluids given in excess of that required, or fluid-transfusions extending into the
 164 re-absorptive phase may cause fluid overload. There is insufficient evidence to justify the use of corticosteroids
 165 in managing dengue shock-syndrome. 42

166 Thrombocytopenia, endothelial-activation, liver-dysfunction and disseminated intravascular coagulation (DICC)
 167 associated with severe dengue, all contribute to bleeding in dengue. Minor bleeding like gum-bleeding and skin-
 168 petechiae are relatively common in dengue fever. These are usually self-limiting and insignificant.
 169 Gastrointestinal (GI) bleeding, menorrhagia, epistaxis and haemoptysis may be severe and require blood-
 170 transfusion. Often, the bleeding may be occult. Significant occult-bleeding should be suspected if there are signs
 171 of intravascular hypovolemia without elevation of haematocrit. Cool peripheries, prolonged capillary refill-time

172 (normal less than 2 seconds), tachycardia and reduced pulse-volume, besides confusional-state are all suggestive
173 of poor circulatory-volume and bleeding.

174 Prolonged-shock and metabolic-acidosis lead to severe bleeding and DIVC. In such cases, packed blood-cells,
175 blood-component or fresh whole-blood transfusion may be required. Prophylactic platelet or fresh frozen
176 plasma-transfusion have not been shown to produce a sustainable rise in platelet-count nor improvement in
177 coagulation profile. 43 - 49 There is also an increased potential-risk of allo-immunization and transfusion-
178 reaction.

179 Other than plasma-leakage and bleeding, dengue virus also has pathogenic-effects on the liver, heart and central
180 nervous-system. Elevations of liver-enzymes are usually mild and are common in dengue. 50 Aspartate
181 aminotransferases (AST) is usually more severely elevated compared to alanine aminotransferases (ALT). The
182 relatively frequent isolation of dengue virus from liver-tissues of fatal-cases of dengue-hepatitis suggests that
183 liver-injury is directly mediated by dengue virus infection of hepatocytes and Kupffer-cells.

184 Haemophagocytic lymphohistiocytosis (HLH) is a rare, but is a potentially fatal medical-condition that can
185 occur after a patient has had severe dengue-infection. Haemophagocytic lymphohistiocytosis is characterized by
186 hyper-inflammation, uncontrolled-proliferation of activated-lymphocytes, prolonged fever, pancytopenia,
187 jaundice and hepato-splenomegaly. However, dengue associated HLH may be under-recognized due to
188 overlapping signs and symptoms of HLH and dengue (e.g., fever, hepato-splenomegaly and pancytopenia).
189 Identification of dengue-patients with, or at risk of developing, HLH is by detection of markedly-high serum-
190 ferritin levels (≥ 500 mg/l) or serum IL2-receptor (≥ 2400 units/L). Signs and symptoms associated with HLH
191 include hepatomegaly, splenomegaly and lymphadenopathy. Dengue with HLH usually has more prolonged
192 fever and hospitalization and is associated with warning-signs. Laboratory-findings include anemia, besides
193 raised aspartate transaminase and alanine transaminase. Treatment includes high-dose corticosteroids and IVIG
194 with or without etoposide. 51

195 Neurological-complications in dengue range between 0.5% and 5.4%, and present in a wide-spectrum of
196 clinical-conditions. 52 - 54 Most commonly it presents as encephalopathy and encephalitis. Other reported
197 manifestations include meningo-encephalitis, intracranial-bleed, transverse-myelitis and Guillain-Barre
198 Syndrome.

199 Despite being a leading cause of illness and mortality in the tropics and subtropics, there are no therapeutics
200 currently available. There is an urgent need to develop an effective antiviral. 55 Newer therapeutic-approaches
201 include direct viral-inhibitors and modifiers of virus-host interactions. 56 - 57 Direct-acting agents include
202 small-molecule inhibitors of essential viral-enzymes (NS2B-3 protease, NS3 helicase, NS5 methyl-transferase,
203 the NS5 polymerase) or small-molecule and antibody-inhibitors of viral entry/fusion.

204 Randomized-trials using chloroquine, lovastatin, balapiravir (a polymerase inhibitor), and celgosivir (an alpha-
205 glucosidase inhibitor) among adults with dengue have not shown a significant benefit on viraemia, NS1
206 antigenaemia, or fever. 58 - 60

207 **Laboratory Diagnosis**

208 Dengue can be diagnosed clinically. However, laboratory-tests are required to confirm the diagnosis. Definitive-
209 diagnosis is important not only for clinical-management of patients, but also for epidemiological-surveillance
210 and monitoring. Development of assays are ongoing and newer assays using more advance technologies have
211 been developed, though not fully validated. 61

212 The pathogenesis of dengue has not been quite completely understood. Moreover, multiple sequential infections
213 occur in dengue endemic areas. Besides, primary and secondary infection-status further cause difficulty in
214 laboratory-diagnosis. 62

215 Improved disease-management requires an effective diagnostic-assay that can be used with confidence -
216 especially in the acute early-stage and for detecting signs of severity. 63

217 The ideal dengue diagnostic-test would be one that is simple, rapid, and affordable having high sensitivity and
218 specificity - which is able to differentiate between primary and secondary infections, and be able to serotype the
219 virus. The time-frame for diagnosis is from the onset to ten days post-disease, remembering that about 2% do
220 not seroconvert. 64

Diagnostic-tools currently are mainly serological-based, nucleic acid-based or antigen-detection. 65

The best diagnostic is isolation of the virus or detection by direct immunofluorescence (IF), or genome-amplification via PCR (66), or detection of viral-antigens like NS1 using either ELISAs or rapid lateral-flow tests. 67 - 68

Very often antibodies are sought for out of convenience, but these are useful diagnostics only when results are obtained using paired samples. Currently, virus-isolation, PCR and Direct IF are done only in reference- and research-laboratories. 64

The NS1-test is more user-friendly. Apart from ELISAs, rapid-flow tests have been developed and validated. 69 - 70

These are useful in the first five days of symptoms when viraemia still exists. Serology is also useful in demonstrating sero-conversion from negative to positive IgM-antibody, or a four-fold increase in IgG antibody-titres in paired serum-specimens. Patients who are IgM-positive and are PCR-negative are usually classified as probable recent dengue-infection, since IgM-antibodies to dengue remain elevated for 90 days after the illness, and could have been from an infection that occurred between two and three months ago. 64

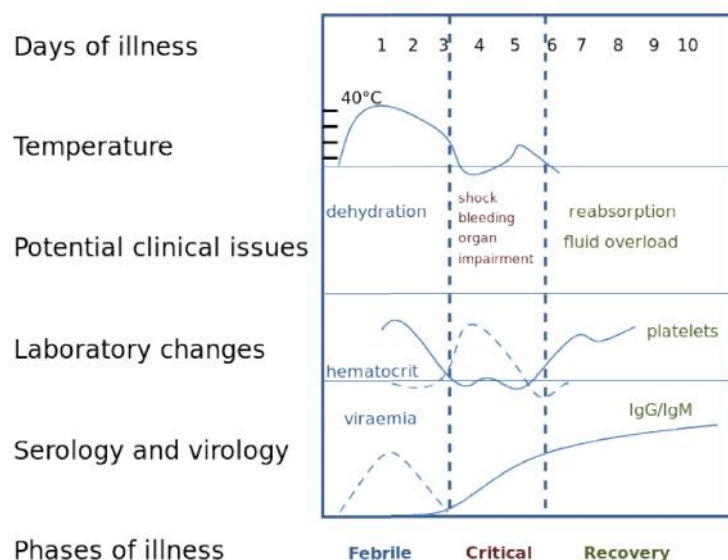
In addition, it is important to note that cross-reactivity with other flavi-viruses, including West Nile virus (WNV), St. Louis encephalitis virus (SLE), Japanese encephalitis virus (JEV), Zika virus and yellow fever virus (YFV), do occur. However, these infections are very rare in Malaysia. Yet, a look into the patient's Recent Travel History would be helpful. A paired-sample, testing for all of the flavi-viruses, best provides diagnosis as to which flavi-virus it is, especially in those who present late after onset of illness (> 5 days), when virus and viral antigens become undetectable. 63, 64

Immune responses to dengue

Dengue virus induces the production of all classes of antibodies, primarily targeting the virus envelope-proteins. The level of antibodies primarily depends on whether the individual has a primary or a secondary dengue infection. 61, 65

Figure 4 shows the timelines of Laboratory Changes viz-a-viz Clinical-features. 26

Figure 4: Timeline of Laboratory Changes viz-a-viz Clinical-features. 26



250 In a primary infection, low antibody-titres are observed - while in a secondary infection, high IgG is detectable
 251 in the acute phase which sometimes masks the detection of IgM in secondary infection. 26

252 In a primary infection, viraemia is present through the incubation-period till the 4th Day of illness. During this
 253 viraemia-phase virus-isolation and virus-RNA detection is possible. NS1 Antigen detection is detectable through
 254 the incubation-period till the 12th Day (convalescent) of the illness. 26

255 IgG and IgM start rising from the 2nd Day. IgM starts falling after the 10th to 11th Day – detectable window-
 256 period occurring from 6th Day to about 30th Day. IgG becomes detectable from about 10th to 11th Day, and
 257 remains detectable, although peaking on about the 15th Day only. 26

258 In secondary-infection, IgG is markedly elevated and remains elevated. IgM is not as markedly elevated as in
 259 primary infection. 26

260 **Table 1. Current Diagnostic-tests for Dengue**

	Procedure	Description
1.	Real-time RT PCR 66	a. Positive result is definite proof of current infection b. Usually confirms serotype c. Where viral-load is high, virus can be isolated d. Negative PCR result should be treated as indeterminate, to be confirmed by a second sample using another marker (or quantitatively via serological tests);
2.	Quantitative or Semi-quantitative Serological-tests 71 - 72	a. Detect IgM and IgG which are best useful when conducted in pairs. b. Many commercially-available such tests. c. Such pairs enable determination of primary and secondary status as well
3.	NS1 ELISA 67 – 68, 73	a. A useful tool b. This test is 100% sensitive
4.	The Plaque Reduction Neutralization Test (or the Fluorescent Reduction Neutralization) (PRNT/FRNT) 64	a. Still the most sensitive serological-test b. But not indicative of the total antibody-levels because non-neutralizing antibodies are not detected. c. Indicates the level of protective antibodies d. labour-intensive, time-consuming, expensive and requires good standardization
5.	The Haem-agglutination Inhibition assay 74	a. has been the gold-standard for many years b. requires a paired-sample – and, skill in interpreting

		the results.
		C. tedious, time-consuming and usually conducted by reference laboratories only

261

262 In addition, a Microfocus-reduction Neutralization-test (based on the peroxidase/anti-peroxidase or fluorescein
263 technique) and a Micro-neutralization Test based on an ELISA-format have been developed.

264 The development of rapid-assays in recent years has allowed patient-specimens to be tested at point-of-care
265 situations. Most manufacturers of these claim that the tests are able to differentiate between primary and
266 secondary dengue-infections. 75 - 76

267 Many biosensors are being developed as rapid-tests for dengue. They use piezoelectric, optical or electro-
268 chemical methods. 77 - 78

269 More recently nanoparticle-beads, mass-spectrometry and micro-sequencing have been utilized and appear
270 promising. 79 - 80

271 These rapid-tests must be properly validated.

272 Future dengue-assays should not only confirm serotype of virus but also have markers that are able to predict
273 disease-severity and indicate level of immunity/protection.

274 **Prevention and Control**

275 The present National Dengue Strategic Plan (2015 – 2020) comprises of 17, 81:

- 276 1. To strengthen the preparedness and response capacity so as to detect cases and outbreaks for an
277 immediate action, and
- 278 2. The National Strategy is developed based on the SWOT analysis as well as the document of "Global
279 Strategy for Dengue Prevention and Control 2012-2020" by WHO

280 The four components of a SWOT analysis examine (a) the current strengths that should be maintained and built
281 on, (b) the weaknesses that need to be addressed, (c) the opportunities that are available for moving toward more
282 optimal function, and (d) the threats that may prevent progress from being made (Blayney DW, 2008).

283 **Table II. The Seven Strategies under the National Dengue Strategic Plan 2015 – 2020. 17, 101**

	Strategy	Description
1.	Dengue Surveillance	1. Includes eNotification since it is a notifiable-disease under the Prevention and Control of Infectious Diseases Act, 1988 17, 81, 82 2. Laboratory Surveillance 17, 81, 82 3. Dengue Outbreak Management 17, 81, 82 4. New breeding-sites 3,9 – 10, 17, 81, 82 5. Strengthening information system 3,9 – 10,17, 81 6. Legislation – heavier penalties 3,9 – 10,17, 81, 82 7. Strengthening Community Participation and Inter-sectoral Collaboration 3,9 –

		10,17, 81, 82 8. Changing insecticide fogging formulation 3,9 – 10,17,101, 102 9. Mass abating 3,9 – 10,17, 81 10. Reducing case-fatality 3,9 – 10,17, 81
2.	National Cleanliness Policy (A) and Integrated Vector Management (IVM) (B) 9 – 10,17, 81, 82	A. 1. The National Cleanliness Policy – holistic and integrated approach through Concept of Inter-agency Blue Ocean Strategy 2. Focus on Clean Environment – Malaysia to become among cleanest countries, free from Infectious Diseases B. 1. Space spraying using Temephos EC or Bti in the hotspot areas 2. Residual spraying as a complementary measure 3. Effective waste collection system 4. Reliable water-supply system to reduce the need for additional water-storage 5. Cleanliness activities (Gotong Royong) 6. Advice on personal protection 7. Inter-agency enforcement at Construction-sites
3.	Management of Dengue Cases	See Section: Clinical Management
4.	Social Mobilization and Communication for Dengue	1. Community-involvement as a COMBI-volunteer 3,9 – 10,17, 81, 82 2. Communication through Mass-media and Social-media 3,9 – 10,17, 81, 82
5.	Dengue Outbreak Response 17-22, 81, 82	1. Epidemic Preparedness Plan: Dengue Outbreaks Operation Room at District and National-level; Inter-agency District Dengue Outbreak Committee chaired by DO; Dengue Task-force Committee at State and National-level 2. Early Detection of Epidemic and Response 3. Risk Communication
6.	Dengue Research 17 – 22, 81	1. Focus on enhancing effectiveness, cost-effectiveness, sustainability and scale of existing interventions 2. Ideas and new methods 3. Collaboration with National Public Health Laboratory (NPHL) and Institute for Medical Research besides other agencies
7.	Reduction of Dengue Burden in	1. Strengthening cooperation and collaboration between State Government, local

	Klang Valley where 57% of total dengue cases occur 17 – 22, 81, 82	authorities and other agencies
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284

285 In Malaysia, the current new directions in dengue-control include 17:

286 1. Having all registered dengue cases confirmed by laboratory tests,

287 2. Increasing source-reduction activity, and

288 3. Reducing fogging-activity from two cycles to one cycle

289 New Tools and Strategy in the Prevention and Control in Malaysia consist of:

290 1. New strategies in hotspot areas such as in Table III:

291 **Table III. New strategies in hotspot areas (including concepts)**

	Strategy	Description/constraints
1.	Residual spraying 17, 81	Read within Text outside this Table
2.	Larviciding activity using Temephos EC or Bti 9-10,17, 81, 82	Read within Text outside this Table
3.	Use of newer-generation insecticides 9-10,17, 81	Read within Text outside this Table
4.	Use of novel ovi-traps and other innovative methods 9-10,17, 81	Read within Text outside this Table
5.	Release of genetically-modified Aedes (A) or Wolbacchia-infected Aedes (B) 9-10, 83, 88 - 89	A. 1. Hampered by logistical-difficulty due to flight-range of Aedes viz-a-viz release-radii in heavily built-up areas B. 1. Same difficulty does not exist because Wolbacchia-infection passed onto progenies – should be self-propagating, but in practice such propagation not found more than 100 meters per year 2. The strain of Wolbacchia shown to be effective in this method not able to survive ambient temperatures in the tropics. This claim is controversial. 3. The Method is undergoing pilot-study by IMR in Selangor

6.	Larviciding of primary water-sources such as water-treatment plants (A), together with Aerial-spraying (B) 82, 84, 86 – 87, 91 - 92	A. 1. Similar to fluoridation of water 2. Pyriproxyfen (after EIA done) or Bti 3. Was done in parts of Brazil B. 1. Aerial-spraying using US CDC Protocol C. Combined method can be implemented if safe and cost-effective vaccine still not found, after pilot-study
7.	Isolation of Cases	1. Not useful since 70-80% of infections are asymptomatic, yet infective 15 – 16, 82, 84 - 87 2. Besides, diagnosis is usually made on 3rd to 5th day 1 - 11

292

293 For the effective control of the dengue-vector mosquito-populations, there is a need to combine several
 294 strategies, such as chemical, biological and integrated control. The chemical-insecticide is the most commonly
 295 used, as it is effective against both the larval and the adult mosquitoes. 85

296 The ultimate aim of insecticide-control management has two parts i.e. the control of Aedes-immatures and the
 297 control of the adult-mosquitoes. The control of adults especially in a dengue-epidemic situation is aimed at the
 298 killing of the infective female-mosquitoes. Whereas, the control of Aedes-immatures is targeted towards the
 299 overall reduction of the mosquito population-density and, in directly reducing the human-vector pathogen-
 300 contact to prevent dengue-transmissions. 85

301 One of the two common chemical-controls are Larvicide-application to treat household drinking-water
 302 containers which has low-relative toxicity, and is safe for humans. The other technique is space spraying (Ultra
 303 low volume, ULV or thermal fogging), which is generally employed in emergency outbreaks of dengue. 86

304 Different chemical groups have been used to control *Aedes spp* in Malaysia since the 1960s, including organo-
 305 chlorines, organo-phosphates, carbamates and pyrethroid-insecticides. The main contributor towards today's
 306 raging dengue epidemics is the worst practice of routine space-spraying and thermal-fogging which do not kill
 307 100% of the vector-mosquito populations. This causes an artificial-selection due to ignorance that has led to
 308 serious chemical-resistance nowadays – and, have seriously impacted human-health following the excessive
 309 uses of various insecticides more routinely and at a higher-frequency. 86

310
 311 The routine space-spraying and fogging pollutes the environment and the food chain - besides also directly
 312 eliminating most of the natural-enemies of the vector-mosquitoes, such as ants, spiders, dragonflies, praying-
 313 mantises, lizards, frogs, birds and bats. These natural-enemies are of great-value as vital biological-control
 314 agents in suppressing the mosquito-populations, which control-measures must be preserved for long-term
 315 successful mosquito-control. 86

316
 317 In view of the above-mentioned predicament, there are innovative new-strategies developed specifically to
 318 outsmart the dengue-vector mosquito. These are described in Table IV. 86

319
 320 **Table IV: Innovative New-strategy in Dengue-vector Control**

321

	Method	Description
1.	Attractant Toxic Sugar Baiting 86	a. Attracts all the hungry and dehydrated adult-mosquitoes (male & female) when they emerge from pupae (especially first two days)

		b. Since nectar-meals are scarce indoors, the bait is the most readily-available and attractive choice. c. Only needs placement in strategic-locations indoor d. Safe because no chemicals extruding into air or environment e. Mostly used as supplement in control
2.	Attractive Lethal Ovi-position Traps 86	a. Makes full-use of the Aedes-vector mosquito's skip-ovi-position characteristic i.e. in using the female as mechanical-vectors to cross-contaminate the other breeding-sites which are beyond our detection. b. Attracts gravid-mosquitoes to come and lay eggs in the special-station that contains water and a lacing-formulation of oviposition-attractants. All (100%) of these eggs cannot develop into adult-mosquitoes. c. The formulation has the insect growth-regulator, IGR, which contaminates these female-mosquitoes - when they lay eggs in their hidden breeding-sites in the wild, they go on to cross-contaminate all the breeding-sites, and all the hidden-eggs. d. All of the chemicals used in this, always stay inside the station - thus protecting all the natural-enemies of the mosquito and ensuring sustainable natural biological-control

In the prevention and control of dengue-outbreaks, the use of household insecticide-products (HIP), such as the insecticide aerosol-sprays have been very much a part of active and sustainable community-participation. 87

They are handy and fast-action, effective in killing all the mosquitoes and always read-to-use. The dengue-hotspot communities should pro-actively do thorough spraying in the morning and in the evening every day within their premise, to ensure that there is no infective female Aedes-mosquitoes hiding within. For non-hotspot communities, such thorough-spraying needs to be done only once a week to ensure there is no Aedes-mosquitoes breeding inside their premise. Therefore, this aerosol insecticide-spray should be integrated into the overall dengue-vectors control-program for maximum control-results. 87

However, those ordinary aerosol-insecticide come with a choking smell, cause staining and leave an oily-film on surfaces, dissuading many. The new generation of mini-aerosol spray-insecticide, equipped with metered-valve and slow-release nano-technology formulation (active ingredient is meto-fluthrin at 0.76%w/w) has been developed to overcome all these negative aspects – they are odorless, clean and dry, very low-volatile organic-compounds (VOCs), non-oily, non health-hazard and eco-friendly compared to the ordinary aerosol-insecticide. 87

In the standard room of up to 30 m³, one just needs to spray at the four corners. Each 83ml (50g) mini aerosol-spray can deliver the fixed-amount in 800 sprays of protection in 200 rooms, providing mosquito-free protection for up to 8 hours. In comparison, the ordinary 600ml (380 g) aerosol-spray could only spray up to 42 rooms and provide an hour of mosquito-free protection. For smaller spaces like in a car, this mini spray can be sprayed once in the car to ensure no mosquitoes while driving. In this manner, it is also a great tool in preventing mosquitoes being transported from one location to another. Outside-fogging can also be done. 87

2. Specific protection

Primary Prevention of diseases classically comprises of Health Promotion and Specific Protection. 84, 88 – 90, 96 Health Promotion has been extensively outlined above. Specific Protection should comprise of an appropriate Mass Vaccination Program of Endemic Areas, appropriate use of effective mosquito-repellents such as DEET, lemon eucalyptus or picaridin, and the appropriate use of mosquito-nets by day-sleeping children, the elderly and the infirm. The last two can be made available, subsidized, at Health Clinics throughout the country. 1 – 10, 84, 88 – 90

In late 2015 and early 2016, the first dengue vaccine, Dengvaxia (CYD-TDV) by Sanofi Pasteur, was registered in several countries for use in individuals 9-45 years of age living in endemic areas. But overall, the much waited-for dengue-vaccine has been a disappointment both in its efficacy and its safety. 97 - 101

If a sufficiently effective and safe vaccine can be found, it will transform dengue fever into a vaccine-preventable disease, and the disease can be quickly brought to near-eradication levels just like all other previous vaccine-preventable diseases.

Takeda Pharmaceutical Company Limited, (“Takeda”) in November 2017 announced the data from an 18-month interim analysis of the ongoing Phase 2 DEN-204 trial of its live, attenuated tetravalent dengue vaccine-candidate, TAK-003 (also referred to as TDV). This interim-analysis showed that children and adolescents who received TAK-003 had a relative-risk of symptomatic-dengue of 0.29 (95% CI: 0.13–0.72) compared to children and adolescents in the placebo control-group. 102

TAK-003 was found to be safe and well-tolerated in terms of solicited local-reactions and systemic adverse-events, relative to the placebo control-group. 97

In participants who were sero-negative at baseline, a second-dose given at Month 3 improved the tetravalent sero-positivity rate at Month 6 to 86%, compared to 69% in the one-dose group. A booster dose at Month 12 resulted in a 100% tetravalent sero-positivity rate at Month 13 in participants who were sero-negative at baseline. 102

TAK-003 is currently under evaluation in the Tetravalent Immunization against Dengue Efficacy Study (TIDES), a large-scale Phase 3 efficacy-trial being conducted in eight dengue-endemic countries. Data from TIDES will be available in late 2018. 102

The US National Institute of Allergy and Infectious Diseases (NIAID) has developed the LATV dengue vaccines TV003/TV005. A single dose of either TV003 or TV005 induced sero-conversion to four DENV serotypes in 74-92% (TV003) and 90% (TV005) of flavivirus-seronegative adults and elicited near-sterilizing immunity to a second dose of vaccine administered 6-12 months later. 103 - 105

The Phase III clinical-trial of the TV003 commenced in February 2016 among 17,000 volunteers in multiple locations in Brazil with the aim of determining its efficacy and safety. The estimated primary-completion date is June 2018, and the estimated study-completion date is December 2022. 103 – 105

When vaccines are available which afford greater than 90% protection against all four strains, the risk of antibody-directed enhancement (ADE) in subsequent natural-infections, causing severe dengue, becomes remote because secondary infections would be rare. Dengue fever very likely will become reduced to sporadic-outbreaks of mostly the Sylvan-type, just like yellow-fever, once a successful mass-vaccination program of a safe and highly-effective vaccine becomes feasible and affordable.

References:

1. WHO. Dengue and severe dengue. April 2017.
2. US Centers for Disease Control. Dengue. 19 Jan 2016.
3. Bains S. Severe Dengue Infection. Medscape. Oct 12, 2017.
4. WHO. Dengue control. 2018.
5. WHO REGIONAL OFFICE FOR THE WESTERN PACIFIC. THE DENGUE STRATEGIC PLAN FOR THE ASIA PACIFIC REGION. Fifty-ninth session 25 July 2008 Manila, Philippines. 22–26 September 2009.
6. WHO Western Pacific Region. WHO ASEAN Workshop on Priority Actions for Dengue Prevention and Control. 3 to 5 May 2011. Manila, Philippines
7. WHO Regional Office for South-east Asia. Comprehensive guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever. 2011
8. WHO. DENGUE GUIDELINES FOR DIAGNOSIS, TREATMENT, PREVENTION AND CONTROL. WHO/HTM/NTD/DEN/2009.1. Expiry date: 2014
9. Song-Quan Ong. Dengue Vector Control in Malaysia: A Review for Current and Alternative Strategies. Sains Malaysiana. 2016. 45(5): 777–785
10. Ang Kim Teng and Satwant Singh. Epidemiology and New Initiatives in the Prevention and Control of Dengue in Malaysia. Dengue Bulletin – Vol 25, 2001
11. Kumarasamy V. Dengue Fever in Malaysia: Time for Review?. Med J Malaysia. Vol 61 No. (1) March 2006
12. Paupy C, H. Delatte, L. Bagny, V. Corbel, D. Fontenille. Institute Pasteur. Aedes albopictus, an arbovirus vector: From the darkness to the light. Microbes Infect. 2009 Dec; 11 (14-15):1177-85
13. Whitehorn J, Kien DT, Nguyen NM, Nguyen HL, Kyrylos PP, Carrington LB, *et al.* Comparative Susceptibility of Aedes albopictus and Aedes aegypti to Dengue Virus Infection After Feeding on Blood of Viremic Humans: Implications for Public Health. J Infect Dis. 2015 Oct 15; 212 (8):1182-90.
14. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, *et al.* Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis. 2000 Jan;181(1):2-9
15. Alera MT, Srikiatkachorn A, Velasco JM, Tac-An IA, Lago CB, Clapham HE, *et al.* Incidence of Dengue Virus Infection in Adults and Children in a Prospective Longitudinal Cohort in the Philippines. PLoS Negl Trop Dis. 2016 Feb 4;10(2):e0004337.
16. Kosasih H, Alisjahbana B, Nurhayati, de Mast Q, Rudiman IF, Widjaja S, *et al.* The Epidemiology, Virology and Clinical Findings of Dengue Virus Infections in a Cohort of Indonesian Adults in Western Java. PLoS Negl Trop Dis. 2016 Feb 12;10(2):e0004390.
17. Zailiza Suli. Dengue Prevention and Control in Malaysia. International Conference on Dengue Prevention and Control & International Dengue Expert Consultation Meeting. December 7-8 , 2015. The Magic School of Green Technologies, National Cheng Kung University, Tainan City. Ministry of Health Malaysia
18. Shepard DS, Undurraga EA, Lees RS, Halasa Y, Lum LC, Ng CW. Use of multiple data sources to estimate the economic cost of dengue illness in Malaysia. Am J Trop Med Hyg. 2012 Nov;87(5):796-805.
19. P. Raviharmman Packierisamy, Chiu-Wan Ng, Maznah Dahlui, Jonathan Inbaraj, Venugopalan K. Balan, Yara A. Halasa *et al.* Cost of Dengue Vector Control Activities in Malaysia. Am J Trop Med Hyg. 2015 Nov 4; 93(5): 1020–1027.
20. Nor Azila, Sharifah Azura Salleh, Hui-min Neoh, Syed Zulkifli Syed Zakaria and Rahman Jamal. Dengue epidemic in Malaysia: Not a predominantly urban disease anymore. BMC Research Notes. 2011, 4:216.
21. Ministry of Health Malaysia. Health Ministry: Dengue cases down in 2017 compared to 2016. The Star Online. 10 Jan 2018.
22. Ministry of Health Malaysia. Almost 83,000 dengue cases nationwide in 2017; 171 deaths. New Straits Times online. 7 April 2018.

- 443 23. Wiwanitkit V. Dengue fever: diagnosis and treatment. *Expert Rev Anti Infect Ther.* 2010
444 Jul;8(7):841-5.
- 445 24. Lai YC, Chuang YC, Chang CP, Lin YS, Perng GC, Wu HC, *et al.* Minocycline suppresses
446 dengue virus replication by down-regulation of macrophage migration inhibitory factor-
447 induced autophagy. *Antiviral Res.* 2018 May 9;155:28-38.
- 448 25. Nitsche C. Strategies Towards Protease Inhibitors for Emerging Flaviviruses. *Adv Exp Med*
449 *Biol.* 2018;1062:175-186.
- 450 26. Academy of Medicine of Malaysia - Clinical Practice Guidelines. 2014, Recommendations
451 For Patient Safety And Minimal Monitoring Standards During ... 2015, Management of
452 Dengue Infection in Adults (3rd Edition).
- 453 27. Rigau-Perez JG, Laufer MK. Dengue-related deaths in Puerto Rico, 1992-1996: Diagnosis and
454 clinical warning signs. *Clin Infect Dis.* 2006 May 1;42(9):1241-6.
- 455 28. Ong A, Sandar M, Chen MI, Sin LY. Fatal dengue haemorrhagic fever in adults during 9. A
456 dengue epidemic in Singapore. *International Journal of Infectious Diseases.* 2007
457 May;11(3):263-7.
- 458 29. Wichmann O, Hongsiriwon S, Bowonwatanuwong C, Chotivanich K, Sukthana Y,
459 Pukrittayakamee S. Risk factors and clinical features associated with severe dengue infection
460 in adults and children during the 2001 epidemic in Chonburi, Thailand. *Trop Med Int Health.*
461 2004 Sep; 9 (9) : 1022-9.
- 462 30. Simmons CP, *et al.* Current concepts: Dengue. *N Engl J Med.* 2012 Apr;366:1423-32
- 463 31. WHO (2009). Dengue: guidelines for diagnosis, treatment, prevention and control - new
464 edition. WHO, Geneva 2009
- 465 32. Taraphdar D, Sarkar A, Mukhopadhyay BB, Chatterjee S. A comparative study of clinical
466 features between monotypic and dual infection cases with Chikungunya virus and dengue
467 virus in West Bengal, India. *Am J Trop Med Hyg* 2012; 86:720.
- 468 33. Lee VJ, Chow A, Zheng X, Carrasco LR, Cook AR, Lye DC, Ng LC, Leo YS. Simple clinical
469 and laboratory predictors of Chikungunya versus dengue infections in adults. *PLoS Negl Trop*
470 *Dis* 2012; 6:e1786.
- 471 34. Mohd Zim MA, Sam IC, Omar SF, Chan YF, AbuBakar S, Kamarulzaman A.. Chikungunya
472 infection in Malaysia: comparison with dengue infection in adults and predictors of persistent
473 arthralgia. *J Clin Virol* 2013; 56:141.
- 474 35. Nimmannitya S. Clinical spectrum and management of dengue haemorrhagic fever. *Southeast*
475 *Asian J Trop Med Pub Hlth.* 1987; 18(3):392-7.
- 476 36. Gubler DJ. Dengue and Dengue Haemorrhagic Fever. *Clin Microbio Review.* 1998; 3.
477 11(3):480-96.
- 478 37. Maria G. Guzmán, Mayling Alvarez, Rosmari Rodriguez, Delfina Rosario, Susana Vázquez, Luis Val
479 dés. Fatal dengue hemorrhagic fever in Cuba, 1997. *Int J Infect Dis* 1999; 3:130–5.
- 480 38. Angeli Ambayya, Anselm Ting Su, Nadila Haryani Osman, Nik Rosnita Nik-Samsudin,
481 Khadijah Khalid, Kian Meng Chang, *et al.* Haematological reference intervals in a
482 multiethnic population. *PLoS One.* 2014 Mar 18;9(3):e91968
- 483 39. Ngo NT, Cao XT, Kneen R, Wills B, Nguyen VM, Nguyen TQ *et al.* Acute management of
484 dengue shock syndrome: a randomized double-blind comparison of 4 intravenous fluid
485 regimens in the first hour. *Clin Infect Dis* 2001; 32:204.
- 486 40. Dung NM, Day NP, Tam DT, Loan HT, Chau HT, Minh LN *et al.* Fluid replacement in
487 dengue shock syndrome: a randomized, double-blind comparison of four intravenous-fluid
488 regimens. *Clin Infect Dis* 1999; 29:787.
- 489 41. Wills BA, Nguyen MD, Ha TL, Dong TH, Tran TN, Le TT, *et al.* Comparison of three fluid
490 solutions for resuscitation in dengue shock syndrome. *N Engl J Med* 2005; 353:877.
- 491 42. Panpanich R, Sornchai P, Kanjanaratanakorn K. Corticosteroids for treating dengue shock
492 syndrome. *Cochrane Database Syst Rev* 2006; :CD003488
- 493 43. WHO. Dengue: guidelines for diagnosis, treatment, prevention and control - new edition.
494 WHO, Geneva 2009
- 495 44. Thomas L, Kaidomar S, Kerob-Bauchet B, Moravie V, Brouste Y, King JP, *et al.* Prospective
496 observational study of low thresholds for platelet transfusion in adult dengue patients.
497 *Transfusion* 2009; 49:1400.

- 498 45. Khan Assir MZ, Kamran U, Ahmad HI, Bashir S, Mansoor H, Anees SB, *et al.* Effectiveness
499 of platelet transfusion in dengue Fever: a randomized controlled trial. *Transfus Med Hemother*
500 2013; 40:362.
- 501 46. Lye DC, Lee VJ, Sun Y, Leo YS. Lack of efficacy of prophylactic platelet transfusion for
502 severe thrombocytopenia in adults with acute uncomplicated dengue infection. *Clin Infect Dis*
503 2009; 48:1262.
- 504 47. Lye DC, Archuleta S, Syed-Omar SF, Low JG, Oh HM, Wei Y, *et al.* Prophylactic platelet
505 transfusion plus supportive care versus supportive care alone in adults with dengue and
506 thrombocytopenia: a multicentre, open-label, randomised, superiority trial. *Lancet* 2017;
507 389:1611.
- 508 48. Lum LC, Abdel-Latif Mel-A, Goh AY, Chan PW, Lam SK. Preventive Transfusion in DSS- is
509 it necessary? *J Pediatr.* 2003 Nov;143(5):682-4
- 510 49. Chairulfatah, A, Setiabudi, D., Agoes, Colebunders R. Thrombocytopenia and platelet
511 transfusions in dengue haemorrhagic fever and dengue shock syndrome. *Dengue Bulletin*,
512 2003;27
- 513 50. Kalayanarooj S, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S, Kunentrasai N, *et al.*
514 Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis* 1997; 176:313.
- 515 51. Esther M Ellis, Tyler M. Sharp, Janice Perez-Padilla, Liza González, B. Katherine Poole-
516 Smith, Emmaculate Lebo, *et al.* Incidence and risk factors developing Dengue-Associated
517 Hemophagocytic Lymphohistiocytosis in Puerto-Rico, 2008 - 2013. *PLOS Neglected Tropical*
518 *Diseases.* 2016. 1 - 14.
- 519 52. Cam BV, Fonsmark L, Hue NB, Phuong NT, Poulsen A, Heegaard ED . Prospective case
520 control study of 127 encephalopathy in children with dengue hemorrhagic fever. *Am J Trop*
521 *Med Hyg* Dec 2001 Dec; 65(6):848–51.
- 522 53. Hendarto SK, Hadinegoro SR. Dengue encephalopathy. *Acta Paediatr Jpn* 1992 128. Jun; 34
523 (3):350–7.
- 524 54. Carod-Artal FJ, Wichmann O, Farrar J, Gascón J. Neurological complications of dengue 129
525 virus infection. *Lancet Neurol.* 2013 Sep; 12(9):906-19.
- 526 55. Low JG, Gatsinga R, Vasudevan SG, Sampath A. Dengue Antiviral Development: A
527 Continuing Journey. *Adv Exp Med Biol.* 2018; 1062:319-332.
- 528 56. Noble CG, Shi PY. Structural biology of dengue virus enzymes: towards rational design of
529 therapeutics. *Antiviral Res* 2012; 96:115.
- 530 57. Krishnan MN, Garcia-Blanco MA. Targeting host factors to treat West Nile and dengue viral
531 infections. *Viruses* 2014; 6:683.
- 532 58. Nguyen NM, Tran CN, Phung LK, Duong KT, Huynh Hle A, Farrar J, *et al.* A randomized,
533 double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue
534 patients. *J Infect Dis* 2013; 207:1442.
- 535 59. Low JG, Sung C, Wijaya L, Wei Y, Rathore APS, Watanabe S, *et al.* Efficacy and safety of
536 celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind,
537 placebo-controlled, proof-of-concept trial. *Lancet Infect Dis* 2014; 14:706.
- 538 60. Whitehorn J, Nguyen CV, Khanh LP, Kien DTH, Quyen NTH, Tran NTT, *et al.* Lovastatin
539 for the Treatment of Adult Patients With Dengue: A Randomized, Double-Blind, Placebo-
540 Controlled Trial. *Clin Infect Dis* 2016; 62:468.
- 541 61. Sekaran, Shamala Devi. Laboratory Diagnosis of Dengue: A Review. *International Medical*
542 *Journal of Malaysia.* 2014; Vol 14 (1): 17-28.
- 543 62. Sekaran S. D. & Soh H. J. Issues in contemporary and potential future molecular diagnostics
544 for dengue. *Expert Review of Molecular Diagnostics.* 2016.
- 545 63. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, *et al.* Dengue: a
546 continuing global threat. *Nat Rev Microbiol* 2010; 8:S7-16.
- 547 64. Sekaran SD, Artsob H. Molecular diagnostics for the detection of human flavivirus infections.
548 *Expert Opinion on Medical Diagnostics.* 2007; 1:521-30.
- 549 65. Mathew A, Rothman AL. Understanding the contribution of cellular immunity to dengue
550 disease pathogenesis. *Immunol Rev* 2008; 225:300-13.
- 551 66. Kong YY, Thay CH, Tin TC, Devi S. Rapid detection, serotyping and quantitation of dengue
552 viruses by TaqMan real-time one-step RT-PCR. *J Virol Methods* 2006; 138:123-30.

- 553 67. Pal S, Dauner AL, Mitra I, Forshey BM, Garcia P, Morrison AC, *et al.* Evaluation of Dengue
554 NS1 Antigen Rapid Tests and ELISA Kits Using Clinical Samples. PloS one 2014;
555 9(11):e113411.
- 556 68. Bessoff K, Delorey M, Sun W, Hunsperger E. Comparison of two commercially available
557 dengue virus (DENV) NS1 capture enzyme-linked immunosorbent assays using a single
558 clinical sample for diagnosis of acute DENV infection. Clin Vaccine Immunol 2008;
559 15:1513–18.
- 560 69. Wang SM, Sekaran SD. Early diagnosis of Dengue infection using a commercial Dengue Duo
561 rapid test kit for the detection of NS1, IGM, and IGG. Am J Trop Med Hyg 2010; 83:690-95.
- 562 70. Wang SM, Sekaran SD. Evaluation of a commercial SD dengue virus NS1 antigen capture
563 enzyme-linked immunosorbent assay kit for early diagnosis of dengue virus infection. J Clin
564 Microbiol 2010; 48:2793-97.
- 565 71. Pal S, Dauner AL, Valks A, Forshey BM, Long KC, Thaisomboonsuk B, *et al.* Multicountry
566 Prospective Clinical Evaluation of Two Enzyme-Linked Immunosorbent Assays and Two
567 Rapid Diagnostic Tests for Diagnosing Dengue Fever. J Clin Microbiol 2014; 53:1092-1102.
- 568 72. Blacksell SD, Jarman RG, Gibbons RV, Tanganuchitcharnchai A, Mammen MP Jr, Nisalak
569 A, *et al.* Comparison of seven commercial antigen and antibody enzyme-linked
570 immunosorbent assays for detection of acute dengue infection. Clin Vaccine Immunol 2012;
571 19:804-10
- 572 73. Guzman MG, Jaenisch T, Gaczowski R, Hang VTT, Sekaran SD, Kroeger A, *et al.* Multi-
573 country evaluation of the sensitivity and specificity of two commercially-available NS1
574 ELISA assays for dengue diagnosis. PLoS neglected tropical diseases 2010; 4:e811.
- 575 74. Clarke DH, Casals J. Improved methods for hemagglutination studies with arthropod-borne
576 viruses. Proc Soc Exp Biol Med. 1955; 88: 96-9
- 577 75. Naz A, Zahid D, Murky SN, Nadeem M, Sil BK, Shamsi TS. Evaluation of efficacy of various
578 immunochromatographic rapid tests for dengue diagnosis. Pak J Med Sci. 2014; 30:166-71.
- 579 76. Ferraz FO, Bomfim MRQ, Totola AH, Ávila TV, Cisalpino D, Pessanha JEM, *et al.*
580 Evaluation of laboratory tests for dengue diagnosis in clinical specimens from consecutive
581 patients with suspected dengue in Belo Horizonte, Brazil. J Clin Viro. 2013; 58:41-6.
- 582 77. Wu TZ, Su CC, Chen LK, *et al.* Piezoelectric immunochip for the detection of dengue fever in
583 viremia phase. Biosens Bioelectron 2005; 21:689-95.
- 584 78. Antje J. Baeumner, Nicole A. Schlesinger, Naomi S. Slutzki, Joseph Romano, Eun Mi Lee,
585 and Richard A. Montagna, *et al.* Biosensor for Dengue Virus Detection: Sensitive, Rapid, and
586 Serotype Specific. Anal Chem. 2002; 74:1442-8.
- 587 79. Fatimah Ibrahim, Tzer Hwai Gilbert Thio, Tarig Faisal. Diagnosis and Management of
588 Tropical Diseases: A Review. Sensors. 2015, 15, 6947-6995;
- 589 80. Antunes, P. *et al.* Quantification of NS1 dengue biomarker in serum via optomagnetic
590 nanocluster detection. Sci. Rep. 2015. 5, 16145
- 591 81. Health Ministry Malaysia. PELAN STRATEGIK PENCEGAHAN DAN KAWALAN
592 DENGGI 2009 – 2013. BAHAGIAN KAWALAN PENYAKIT, KEMENTERIAN
593 KESIHATAN MALAYSIA. 4 FEBRUARI 2009
- 594 82. Meer Ahmad AM. Target real Aedes breeding grounds. The Star Online (Letter to Editor). 7
595 Jan 2017.
- 596 83. Meer Ahmad AM. Questions over dengue control methods. The Star Online (Letter to Editor).
597 8 Nov 2016.
- 598 84. Meer Ahmad AM. Dengue vaccine is still viable. The Star Online (Letter to Editor). 18 Aug
599 2016.
- 600 85. Bisset JA, Rodríguez MM, San Martín JL, Romero JE, Montoya R. Evaluación de la
601 resistencia a insecticidas de una cepa de Aedes aegypti de El Salvador. Rev. Panam. Salud
602 Publ. 2009; 26(3):229-234.
- 603 86. World Health Organization. Vector surveillance and control. In: Dengue Haemorrhagic Fever
604 – Diagnosis, treatment, prevention and control. 2nd ed. Geneva, 1997. p. 48-59.
- 605 87. H. H. Yap, N. L. Chong, A. E. S. Foo and C. Y. Lee. Dengue vector control: Present status
606 and future prospects. Kaohsiung J Med Sci 10: S102--S109, 1994
- 607 88. Meer Ahmad AM. Reduce cost of dengue tests. The Star Online (Letter to Editor). 22 Jan
608 2016.

- 609 89. Meer Ahmad AM. Why shun the dengue vaccine? Free Malaysia Today News Portal. (Letter
610 to Editor) 30 June 2016.
- 611 90. Meer Ahmad AM. A simple strategy to control dengue fever. Free Malaysia Today News
612 Portal. (Letter to Editor) 12 March 2016.
- 613 91. Ross PA, Wiwatanaratnabutr, Axford JK, White VL, Endersby-Harshman NM, Hoffmann
614 AA. Wolbachia Infections in *Aedes aegypti* Differ Markedly in Their Response to Cyclical
615 Heat Stress. *PLoS Pathog*. 2017 Jan 5;13(1): e1006006.
- 616 92. Ye YH, Carrasco AM, Dong Y, Sgrò CM, McGraw EA. The Effect of Temperature on
617 Wolbachia-Mediated Dengue Virus Blocking in *Aedes aegypti*. *Am J Trop Med Hyg*. 2016
618 Apr;94(4):812-9.
- 619 93. Schmidt TL, Barton NH, Rašić G, Turley AP, Montgomery BL, Iturbe-Ormaetxe I, *et al*.
620 Local introduction and heterogeneous spatial spread of dengue-suppressing Wolbachia
621 through an urban population of *Aedes aegypti*. *PLoS Biol*. 2017 May 30; 15(5):e2001894.
- 622 94. Alyssa Navarro. Clearing the Air: Monsanto Breaks Silence On Zika Virus, Microcephaly
623 And Sumitomo Larvicide. *Tech Times*. 16 February 2016.
624 [http://www.techtimes.com/articles/133955/20160216/clearing-the-air-monsanto-speaks-out-](http://www.techtimes.com/articles/133955/20160216/clearing-the-air-monsanto-speaks-out-about-zika-virus-microcephaly-and-sumitomo-larvicide.htm)
625 [about-zika-virus-microcephaly-and-sumitomo-larvicide.htm](http://www.techtimes.com/articles/133955/20160216/clearing-the-air-monsanto-speaks-out-about-zika-virus-microcephaly-and-sumitomo-larvicide.htm)
- 626 95. US Centers for Disease Control. Information on Aerial Spraying; Aerial Spraying and
627 Mosquito Control. 10 October 2017.
- 628 96. “Introduction to Epidemiology” in *A Textbook of Community Medicine in South-east Asia*.
629 Phoon and Chen PCY, Editors. John Wiley & Sons Ltd. 1986
- 630 97. Meer Ahmad AM. Reveal vaccine’s efficacy, safety. *The New Straits Times*. 21 Feb 2017.
- 631 98. Meer Ahmad AM. Doubts in the Efficacy and Safety of the CYD-TDV Dengue-Vaccine.
632 *Berita MMA Newsletter*. March 2017.
- 633 99. Ferguson N, Isabel Rodríguez-Barraquer, Ilaria Dorigatti, Luis Mier-y-Teran-Romero, Daniel
634 J. Laydon, Derek A. Benefits and risks of the Sanofi-Pasteur dengue vaccine: Modeling
635 optimal deployment. *Science*. 02 Sep 2016: Vol. 353, Issue 6303, pp. 1033-1036.
- 636 100. Health Ministry Malaysia. Ministry warns of effects of Dengvaxia. *The Star Online*. Sunday, 3
637 Dec 2017.
- 638 101. Health Ministry Malaysia. Dengvaxia dengue vaccine not distributed in Malaysia, says Health
639 DG. *Malay Mail*. 03 December 2017.
- 640 102. Takeda Newsroom. Takeda’s Dengue Vaccine Candidate Associated with Reduced Incidence
641 of Dengue in Children and Adolescents; New 18-Month Interim Phase 2 Data Published in
642 *The Lancet Infectious Diseases*. 7 Nov 2017.
- 643 103. NIH US National Library of Medicine. Phase III Trial to Evaluate Efficacy and Safety of a
644 Tetravalent Dengue Vaccine. Butantan Institute. *ClinicalTrials.gov*. 13 March 2018.
- 645 104. Whitehead SS. Development of TV003/TV005, a single dose, highly immunogenic live
646 attenuated dengue vaccine; what makes this vaccine different from the Sanofi-Pasteur CYD™
647 vaccine?. *Expert Rev Vaccines*. 2016 Apr; 15(4): 509–517. Published online 2015 Dec 2.
- 648 105. Whitehead SS, Anna P. Durbin , Kristen K. Pierce, Dan Elwood, Benjamin D. McElvany,
649 Ellen A. Fraser, *et al*. In a randomized trial, the live attenuated tetravalent dengue vaccine
650 TV003 is well-tolerated and highly immunogenic in subjects with flavivirus exposure prior to
651 vaccination. *PLOS: Neglected Tropical Diseases*. 8 May 2017.