Dengue: The Breakbone Fever

WRAIR Tropical Medicine Course

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Walter Reed Army Institute of Research
NOT the Band
Disclaimer

• The points made in this presentation are solely the views/opinions of the author and do not reflect the views/opinions of the US Government, US Department of Defense, US Army or the US Army Medical Research and Materiel Command.
Take Home Points

• Mosquito-borne illness
  – Not spread person-to-person
• First infection can be a bad experience
• Second infection can be deadly
• No antiviral treatment
• No vaccine (yet)
Case (1)

- 25 y/o male Indiana Jones type presents to your clinic in June @ Ft. Bragg, NC with c/o headache, abdominal pain, nausea and vomiting for the past 24 hours. Took pepto bismol and tylenol without relief.
- He returned from leave OCONUS 2 days ago (SubSaharan Africa, Latin America and SE Asia) where he swam in the ocean, ate adventurously, suffered numerous different insect bites, partook of some “horizontal refreshment” with local talent, and volunteered to be a cow herder for 2 weeks in the Pampas. He has 2 cats, a dog, tropical fish and several ferrets as pets at home. 3 weeks ago he cleaned out his aquarium, and stated it was a “bloody chore”. He did not take appropriate prophylaxis prior to/during the trip.
- What do you do?
- A) Bellyache, GOMER, discharge
- B) Give him extra-strength PB and discharge with instruction to f/u with primary HCP next week,
- C) Admit, evaluate for, among other things, malaria, dengue and RMSF
- D) Consult psychiatry
Introduction to Dengue

• Definition: Mosquito-borne flaviviral disease.

• Etiology:
  Infection with one of four types of dengue virus:
  – DEN-1
  – DEN-2
  – DEN-3
  – DEN-4

• Transmission:
  – Vector: *Aedes* mosquito
    • *Aedes aegypti*
    • *Aedes albopictus*
  – Blood transfusion
  – Organ transplantation
  – No person-to-person transmission documented
History of Dengue

- Clinical descriptions date as far back as 992 AD in China
- David Bylon (Batvia) in 1779
  - “knokkelkoorts” – joint fever
- Benjamin Rush
  - Termed “breakbone fever”
  - Comes from Swahili “ka dinga pepo” meaning a sudden cramp like seizure and plague

http://www91.homepage.villanova.edu/eli.greenbaum/peru.htm
History of Dengue (2)

- Viral etiology suggested in early 1900’s by Ashburn and Craig
- Virus types 1 and 2 isolated during World War II
- 1956 outbreak in Manila led to identification of Den-3 and DEN-4
- Dengue hemorrhagic fever recognized since 1950’s
Dengue Virus

- Flavivirus (single-stranded RNA virus)
- Spherical, 40-50 nm (dia.) viral particle
  - 3 Structural (E, C, M) proteins
  - 7 Nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5)
- 4 serotypes
  - DENV 1 through 4
  - Multiple genotypes per serotype
- Common progenitor 1,000 years ago
- Serotypes have further divergence
  - 62 to 67% homology based on amino acid sequence
- Varying pathogenicity based on serotype
Case 2

• 50 y/o man with multiple mosquito bites after exploring the Amazon during a recent (2 weeks ago) trip. Had been recently web surfing and found out about dengue. He asks you if he should take prophylaxis against dengue. He has been asymptomatic. What do you do?

• A) Admit, put on ribavirin
• B) Reassure
Vector

- *Aedes aegypti* and *Ae. albopictus*
  - Highly susceptible to dengue
    - Efficient vectors
  - Prefers human blood
  - Daytime feeder: interrupted, between laying
    - 0800-1300; 1500-1700
  - Bite goes unnoticed
  - Multiple bites per blood meal; one mosquito can infect several persons
  - Adapted to urban life; breeds in freshwater containers

- **RAPID TRANSMISSION, EXPLOSIVE EPIDEMICS**
Larvae

A water sample is teeming with mosquito larvae after it was collected from a fountain outside a vacant house July 15 in Miami Beach, Fla. Miami-Dade County health officials are reporting the first suspected local case of dengue fever, a potentially serious mosquito-borne illness that had once disappeared from the United States.

Habitat

- Breeds in clean, still, stagnant water
  - Discarded tires
  - Water tanks
  - Storage appliances

http://www.fcen.uba.ar/habitat/dengue.htm
Breeding sites
Dengue: Epidemiology Assessment

- Leading arboviral (mosquito-borne) infection
- Major health problem in the subtropics and tropics (~35°N and ~35° S)
  - Southeast Asia, India, Middle East, Caribbean, Central and South America, Australia, South and Central Pacific
  - Transmission in ~ 100 countries
  - Recent suspected dengue outbreaks
    - Yemen, Pakistan, Saudi Arabia, Sudan, Cape Verde
Epidemiology Assessment (2)

- 2.5 billion people at risk for infection
- 50-100 million infections annually
- ~500,000 cases of DHF annually
- Up to 25,000 deaths annually
- Significant Economic Burden
  - SE Asia: 1,300 disability-adjusted life years
  - Similar to TB, other childhood and tropical diseases
World distribution of dengue viruses and their mosquito vector, *Aedes aegypti*, in 2005

The tropical zone of the world between 350N and 350S latitude and area not over 1,000 ft. above sea level is the usual habitat, the areas are marked by monsoon-rains.
Total Dengue Cases and Deaths, 2003-2008

Source: DengueNet
The Global Resurgence of Dengue

- Unprecedented global population growth
- Unplanned and uncontrolled urbanization
- Lack of effective mosquito vector control
- Globalization of trade

Aedes aegypti in the Americas (1970), at the end of the mosquito eradication program, & in 2002

American Countries with laboratory confirmed dengue hemorrhagic fever, prior to 1981 and from 1981 to 2003

More man-made breeding grounds (waste)
- Increased international air travel
- Decay in public health infrastructure

Source: WHO/PAHO/CDC, Aug. 2004
Global distribution of dengue virus serotypes, 1970
Air Traffic Global Flight Patterns
Global distribution of dengue virus serotypes, 2004
Dengue in the Americas
1980 – 2007 *

* Data: PAHO (Nov. 30, 2007)

* Data: PAHO (Nov. 30, 2007)
Presence of DEN-3 in the Americas, 1994 -2007

1994-1995
1997-2007
Not reported

## Casos Sospechosos de Dengue Semanal, Año 2010

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Dengue in the USA

• Some historical dengue outbreaks in the USA
  – 1780: Philadelphia, PA
  – 1826-8: Savannah, GA
  – 1850-1: Charleston, SC, Savannah, GA, New Orleans, LA, Mobile, AL, Galveston, TX, Augusta, GA
  – 1922: Texas, Savannah, GA
  – 1934: Florida
  – 1945: New Orleans
Dengue in the USA (2)

• Recent indigenous transmission
  – Texas:
    • 1980: 23 cases, first locally acquired since 1945
    • 1980-1999: 64 cases (lab-documented)
    • 2005: DEN-2 epidemic in Brownsville; estimated incidence of recent dengue infection (4% of population)
  – Hawaii:
    • 2001-2002: 122 cases (first since 1944)
  – Florida (Key West):
    • 2009-2011: 93 cases (as of 17 May 2011)
    • 6 cases to date in 2011: Miami-Dade (2), Palm beach (2), Martin (1), Hillsborough (1)¹ Counties

It is here!

Dengue fever outbreak feared in Key West [Updated]
July 14, 2010 | By Thomas H. Maugh II, Los Angeles Times

Federal officials said Tuesday that they fear an outbreak of dengue fever in Florida after a survey of Key West residents found that at least 5% had been infected or exposed to the virus. With the exception of a handful of isolated cases along the Texas-Mexico border, there had previously been no cases in the continental United States since 1946 and no outbreak in Florida since 1934.

5% of Key West Population Infected in 2009; New Case Suggests Ongoing Outbreak
By Daniel J. DeNoon
WebMD Health News
Reviewed by Laura J. Martin, MD
Key West Dengue

• RT-PCR done on 1,178 pools of *Ae. aegypti* mosquitoes collected from Monroe County, FL from 27 January-17 December 2010

• DENV-1

• KW sequence grouped as a member of a large clade of recent DV from Central America

• Unknown time of introduction into FL
Dengue Impact on U.S. Military Operations

- Philippines
- World War II
- Vietnam

Hospitalized US military personnel, Philippines
Dengue Impact on recent U.S. Military Operations

• Somalia
  – Operation Restore Hope (1993)
  – 58/289 (20%) hospitalized febrile troops had laboratory-confirmed DF
  – 69/289 (24%) suspected DF cases

• Haiti
  – Operation Uphold Democracy (1994)
  – 31/103 (30%) hospitalized febrile troops had DF

• Defense Medical Surveillance System (DMSS)
  – 1997-2006: 26 DF cases hospitalized, 170 ambulatory
USASOC Study

• Seroprevalence study
• USASOC personnel deployed to dengue-endemic areas in Latin America
  – At least 30 days, from 2006-2008
  – 500 specimens
  – DoD Serum Repository
  – Sandwich ELISA
• 11.0% seroprevalence rate
Pathogenesis

• Multiple theories regarding pathogenesis but none accepted

• Lack of a reliable animal model

• Complicated host and viral interactions
  – Different responses in adults and infants

• Antibody dependent viral enhancement
  – Upregulation of infection
  – Increased cytokine activity

• Unknown etiology of capillary leak syndrome characterized by DSS
Pathogenesis (2)

• No evidence of direct viral infection of endothelial cells\(^1\)
• Transient disruption in the function of the endothelial glycocalyx layer
  – A molecular sieve
  – Hypoalbuminemia, proteinuria
  – DENV and NS1 adhere to heparan sulfate
  – Increased urinary heparan sulfate excretion seen in kids with severe dengue\(^2\)

\(^1\) Leong AS et al. Semin Diagn Pathol 2007;24:227-36
Clinical Manifestations

Dengue Shock Syndrome

Dengue Hemorrhagic Fever

Asymptomatic DENV infections

50 to 90% of cases DEN-2 and DEN-4

Clinical Dengue

– Spectrum of clinical illness

• Primarily defined in Thai cohorts
• Asymptomatic infection 50%
• Undifferentiated fever 45%
• Dengue fever
• Dengue hemorrhagic fever (DHF)/ Dengue shock syndrome (DSS) <5%
  – Case fatality rate for DHF <1% with proper medical management; >50% without.
Old Classification of Dengue

Fever → Positive tourniquet test → Increased vascular permeability → Hypovolemia → Shock → Death

Other hemorrhagic manifestations:
- Rising hematocrit
- Hypoproteinemia
- Serous effusion

Leakage of plasma → Hypovolemia

Grade I

Grade II

Coagulopathy

Grade III

Disseminated intravascular coagulation

Grade IV

Severe bleeding

Death

http://lukemaciel.deviantart.com/art/Mosquito-da-Dengue-87552543
Old Definition of Dengue Hemorrhagic Fever

• Fever lasting 2-7 days
• Tendency to hemorrhage
  – Positive tourniquet test (TT)
  – Spontaneous bleeding
• Platelet count <100X10⁹ per litre
• Evidence of plasma leak
  – Increasing hematocrit
  – Pleural effusions
New Classification

- Dengue Fever (DF)
  - Classical DF, recovers without major sequelae
- Severe Dengue
  - Plasma leakage resulting in shock
  - Accumulation of serosal fluid
  - Severe bleeding
  - Severe organ impairment

Most Dengue infections are...

• Asymptomatic, or
• Mild symptomatic illness
  – Undifferentiated fever
  – +/- Rash
Dengue Fever (DF)

- Incubation period 3-7 days, illness lasts ~7 days

- A range of clinical manifestations
  - Three phases
  - Febrile
  - Critical
  - Recovery

- Epi: Travel/residence in urban areas of tropics/sub-tropics

http://professoralilianbiologia.blogspot.com/2008/04/lilian-cia.html
DF (Day 1, Febrile Phase)

- Abrupt onset high fever (≥38.5° C)
  - 5-7 days fever (biphasic)
- Rash
  - Early flushlike rash may be replaced by a macular/morbilliform rash. Late petechiae
- Chills, vomiting
- Arthralgias, myalgias
- Severe headache
- Eye, Retro-orbital pain
- Lumbosacral pain

DF (Day 2, Febrile Phase)

- Severe muscle, joint pain
- Nausea, vomiting
- Lassitude, prostration
- Respiratory symptoms
- Epistaxis, gum bleeding, petechiae
  - Classic DF with some hemorrhage is NOT DHF
- PE:
  - Fever
  - Generalized rash (may be replaced by macular/morbilliform later on). Petechial late
  - Relative bradycardia
  - Generalized lymphadenopathy
  - Petechial hemorrhages
- After 3-7 days, no sequelae
Rash

Case 3

- 27 y/o AD USMC from Puerto Rico presented with 2 days of increasing fever (>38.5 C), severe headache, rash, arthralgias, myalgias, while on deployment in the Philippines. After 5 days of illness, his fever suddenly resolved. Should you:
  - 1) Discharge
  - 2) Draw labs and observe
Symptomatic Dengue Clinical Syndromes

Dengue Fever (DF)

Dengue Hemorrhagic Fever (DHF)

Dengue Shock Syndrome (DSS)
Critical Phase

• Small proportion of patients
  – Children, young adults

• Occurs at time of defervescence
  – Around days 4-7 of illness

• Systemic vascular leak syndrome
  – Increasing hematocrit
  – Hypoproteinemia
  – Pleural effusions
  – Ascites
Severe Dengue

- Severe plasma leakage
  - Shock (DSS)
  - Serosal fluid accumulation with respiratory distress
- Severe bleeding
  - Clinically evident
- Multi-organ involvement
  - Liver: AST/ALT >1000
  - CNS: Impaired consciousness, seizures, encephalopathy
  - CV and other
DHF

http://www.pattayagogos.com/news08a.htm
http://denguehemorrhagicfever.tumblr.com/
Dengue Hemorrhagic Fever (DHF)

- Onset as per classical dengue
- Damage to blood and lymph vessels
- Defervescence followed (2-5 days) by
  - Ascites, abdominal pain
  - Pleural effusion
  - Hemorrhagic manifestations (gum bleeding, phlebotomy bleeding) which may progress to shock
  - Central cyanosis
  - Diaphoresis
- Epi: Exposure in dengue endemic region with possible previous dengue infection
DHF (2)

- PE:
  - Restlessness
  - Abdominal pain
  - Hemorrhage
    - Petechiae
    - Spontaneous ecchymoses
    - Bleeding: GI, GU, phlebotomy
  - Tender hepatomegally (75%), splenomegally
  - Pleural* effusions (80%) perirenal effusions (77%), hepatic, splenic, pericardial, peritoneal effusions* (variable%)
  - Shock
    - Rapid, weak pulse
    - Pulse pressure <20 mmHg
    - Unobtainable BP
Lab

- Positive tourniquet test (or hemorrhagic manifestations)
- Thrombocytopenia (<100,000)*
- Increase in aPTT, decrease in fibrinogen
- Plasma leakage
  - Hemoconcentration (Hct. inc. >20%)*
  - Pleural effusion/ascites
  - Petechiae
- Hepatorenal shutdown with shock
- Viral isolation from acute serum
- Convalescent IgM (+)
- Peak proteinuria**
  - 0.56 v. 0.08 g/d (P<0.001), onset 1 day after defervescence (-2 to 3 days)

Dengue Shock Syndrome (DSS)

- Fluid leak outside of blood vessels
- Lasts 1-2 days
- Massive hemorrhage
- Shock, peripheral vascular collapse
  - Hypoperfusion c/b myocardial dysfunction: metabolic acidosis and MOF
- Cyanosis, massive pleural effusions, ascites
- Narrowing pulse pressures (<20 mmHg)
- Can be fatal (50% in underserved populations; 1% in established centers)
Subcutaneous hemorrhage in DHF
Risk Factors for DHF/DSS

- Pre-existing immunity from previous infection (heterogenous subtype)
- Diabetics, asthmatics, other chronic diseases
- DENV type
  - DENV-1,3 > 2,4
- Increased time between infections
- Under age 15
  - Increased capillary fragility
- HLA type and race*
  - Caucasian>AA
  - HLA Class-1 alleles
- Female sex
- AB blood group
- Promotor variant of DC-SIGN receptor
- Single-nucleotide polymorphism in TNF gene

*De la C Sierra B, Kouri G, Guzman MG. Arch. Virol., 2007, 152(3) 533-42. Epub 2006 Nov. 16.
Factors that reduce the risk of severe dengue

- Race
- Second or third degree malnutrition
- Polymorphisms in the Fc-gamma and Vitamin D receptor genes
Criteria For Dengue +/- Warning Signs

• Probable case:
  – Resident/travel to dengue endemic area and 2 of the following:
    – Nausea, vomiting
    – Rash
    – Aches and pains
    – + TT
    – Leukopenia
    – Any warning sign
Warning Signs

- Continual/increasing abdominal pain/tenderness
- Persistent vomiting
- Clinical fluid accumulation (serosal)
- Mucosal bleeding
- Lethargy, restlessness
- Tender hepatomegaly (>2 cm), ascites
- Lab: increase in Hct. concurrent with rapid decrease in platelets*
- Sudden reduction in temperature
Recovery Phase

• Altered vascular permeability syndrome resolves
  – After 48-72 hours
• Rapid improvement in patient’s symptoms
• Rash
  – Mild maculopapular to severe, pruritic lesions (leukocytoclastic vasculitis)
  – Resolves with desquamation (1-2 weeks)
• Profound fatigue for several weeks
Case 4

• 30 y/o AD Sailor who recently returned from a TDY to Thailand 4 days ago. Has had 2 days of fever, excruciating HA, eye pain, severe myalgias, arthralgias, sweats and rash. You suspect dengue.

• How to diagnose?

• How to treat?
Lab

- Marked leukopenia
- Thrombocytopenia
- Moderate elevation of AST/ALT
- Viral isolation to Day 5 only
- Negative malaria smears
- Dengue IgM (+) on Day 6 serum
  – Takes 5 days to manifest
- PCR available
- Convalescent: 4-fold rise in IgG may be required
Antibody specificity increases over time

Most readily available diagnostic tests
- ELISA (serology)
  - Cross-reactive; not specific
  - MAC-ELISA, IgG ELISA
  - IgM/IgG (>1.2, 1.4?) not defined
- PRNT, microneutralization (serology)
  - More specificity
  - Research, vaccine work
- Viral Isolation
  - Most specific
Lab (3)

- **Nucleic Acid Amplification (NAAT)**
  - RT-PCR
  - Real Time RT-PCR
  - NASBA
  - None commercialized to date
  - None standardized

- **Antigen Detection**
  - NS1
  - Antigen capture ELISA, lateral flow antigen detection, NS1 IgM, IgG responses.
  - Do not differentiate between the different serotypes
Immune Response to Dengue

NS-1: Effective days 1-5 post onset of symptoms

IgM/IgG: Effective after day 5

A diagnostic capable of detecting both is desirable

Slide courtesy of Dr. Subhamoy Pal
• **Primary infection**
  – IgM first to appear, at end of 3-5 day fever period (~50%), day 6-10 (93-99%), peak (2 weeks), undetectable by 2-3 mos.
  – IgG appears at end of first week of illness, persists for >year
  – RT-PCR can provide a same-day diagnosis with a similar sensitivity to culture

• **Secondary infection**
  – IgM typically LOWER titer than primary infection; false negatives have occurred
  – IgG typically HIGHER titer than primary infection; may x-react with other flaviviruses (JE, YF, WN)
Criteria for Confirmed and Probable Dengue Infection

• Confirmed
  – Viral isolation
  – Genome detection
  – Antigen detection
  – IgM/IgG seroconversion

• Probable
  – IgM positive
  – Elevated IgG titer (> 1,280 by HAI)
Diagnosis

Dengue Diagnostic Process

Days Post-onset

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Acute Serum
RT-PCR

- Undetermined cause of illness

+ Identification of DENV-1, -2, -3 or -4

Convalescent Serum

IgM ELISA

- No evidence of recent infection

+ Recent infection

IgM -

IgM +

IgG -/+ 4 x (IgG +)

## Tests Used for the Lab Diagnosis of Primary Dengue Infection

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<td>RT-PCR</td>
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<td>IgG ELISA</td>
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<td>PanBio duoCassette</td>
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<td>Refrigerate if &lt;6 hrs, if &gt;, -20°C</td>
<td>1-2 days</td>
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<td>Serum Neutralization (PRNT)</td>
<td>1 week to &gt;1 year post-infection</td>
<td>1 mL</td>
<td>Frozen or refrigerated</td>
<td>1 week</td>
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*SST or red top tube
Virus isolation in cell culture and detection by IFA
Used with IgG ELISA to differentiate primary from secondary infection
Used with IgM ELISA to differentiate primary from secondary infection*
Rapid Diagnostic Tests (RDT’s)

Important for:

- Quick diagnosis (lab results take time and require labs)
- In resource-limited settings
- Alerts a unit to ID threats
- Helpful for triage during outbreaks
- Curtail geographic spread of infectious diseases
- **Stability operations and infrastructure building**

Worldwide demand for better diagnostics to manage treatment and prevention

Slide courtesy of Dr. Subhamoy Pal
Current Rapid Diagnostic Technologies

- Lateral Flow
- Agglutination
- Flow through
- Solid Phase
- Isothermal Nucleic Acid Tests

Slide courtesy of Dr. Subhamoy Pal
Product Introduction

#1: IgG/IgM Dengue Duo Cassette

10μL of serum, plasma, or whole blood

15 minutes (time to result)

Wu et. al. CDLI 2000, pp 106-109
#2: NS-1/IgG/IgM Dengue Duo Cassette

120μL of serum or plasma
15 minutes (time to result)


Comparison of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection

Lyda Osorio¹, Meleny Ramirez², Anilza Bonelo², Luis A Villa³, Beatriz Parra²

Abstract

Background: We compared the diagnostic accuracy and reproducibility of commercially available NS1-based dengue tests and explored factors influencing their sensitivities.

Methods: Paired analysis of 310 samples previously characterized as positive (n = 218) and negative (n = 92) for viral isolation and/or RT-PCR and/or IgM seroconversion. Masked samples were tested by two observers with Platelia™ Dengue NS1 Ag, second generation Pan-E™ Dengue Early ELISA, SD Dengue NS1 Ag ELISA, Dengue NS1 Ag STRIP™, and SD BIOLINE™ Dengue Duo (NS1/IgM/IgG).

Results: SD BIOLINE™ NS1/IgM/IgG had the highest sensitivity (80.7% 95%CI 75-85.7) with likelihood ratios of 7.4 (95%CI 4.1-13.8) and 0.21 (95%CI 0.16-0.26). The ELISA-format tests showed comparable sensitivities; all below 75%. STRIP™ and SD NS1 had even lower sensitivities (<65%). The sensitivities significantly decreased in samples taken after 3 days of fever onset, in secondary infections, viral serotypes 2 and 4, and severe dengue. Adding IgM or IgG to SD NS1 increased its sensitivity in all these situations.

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Standard Diagnostics Dengue Duo (NS-1) RDT

**NS1 Ag**

3 drops (110 μl) of plasma or serum for early acute phase samples (day 1 ~ 5)

**IgG/IgM Ab**

10 μl of plasma or serum for early convalescence phase samples (after day 5 ~ 14)

Ag/Ab level

Slide courtesy of Dr. Subhamoy Pal
Interpretation

- Negative
- Primary
- Secondary

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Why make a primary/secondary determination?

- The majority (>90%) of DHF/DSS cases are secondary infections
  
  One 20 year longitudinal study suggests that among all DHF/DSS cases 9% are primary and 91% are secondary (Nisalak, A., et al., Am J Trop Med Hyg, 2003. 68(2): p. 191-202)

- Overall, 2-4% of secondary infections proceed to severe dengue. Other risk factors also need to be considered (Guzman, M.G., et al., 1997. Am J Epidemiol, 2000. 152(9): p. 793-9; discussion 804)

Positive predictive value of secondary infection leading to DHF varies by region and attack rate.

Slide courtesy of Dr. Subhamoy Pal
Commercially Available NS-1 Products

• Rapid Tests
  – Bio-Rad Strip™
  – SD (Focus) BIOLINE Dengue NS1 Assay
  – SD (Focus) BIOLINE Dengue Duo IgM/IgG/NS1 Assay
  – Panbio Dengue Early Rapid

• ELISA format
  – Panbio Dengue Early PanE (2nd Generation)
  – SD NS-1 Dengue Ag ELISA
  – Bio-Rad Platelia™ Dengue NS1 Ag (Evaluated by NMRC)

Slide courtesy of Dr. Subhamoy Pal
## Assay specifications

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SD Rapid</th>
<th>Biorad Rapid</th>
<th>Panbio Rapid</th>
<th>BioRad ELISA</th>
<th>Panbio ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of steps</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td><strong>Blood matrices</strong></td>
<td>EDTA-treated blood, plasma, sera</td>
<td>Plasma, sera</td>
<td>Sera</td>
<td>Plasma, sera</td>
<td>Sera</td>
</tr>
<tr>
<td><strong>Assay Time</strong></td>
<td>15-20 minutes</td>
<td>15-30 minutes</td>
<td>15 minutes</td>
<td>140 minutes</td>
<td>160 minutes</td>
</tr>
<tr>
<td><strong>Volume necessary</strong></td>
<td>105 uL</td>
<td>50 uL</td>
<td>50 uL</td>
<td>50 uL</td>
<td>75 uL</td>
</tr>
<tr>
<td><strong>Format</strong></td>
<td>Cassette</td>
<td>Dipstick</td>
<td>Dipstick</td>
<td>96-well</td>
<td>96-well</td>
</tr>
<tr>
<td><strong>Extra materials required</strong></td>
<td>No</td>
<td>Tubes, pipette</td>
<td>Pipette</td>
<td>Pipette, incubator, plate reader</td>
<td>Pipette, incubator, plate reader</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Room Temp.</td>
<td>2-8°C</td>
<td>2-8°C</td>
<td>2-8°C</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>

Slide courtesy of Dr. Subhamoy Pal
Summary of data

Panbio Dengue IgM/IgG Duo Cassette RDT
- Down-selected from among several Dengue RDT’s
- Meets KSA and Attributes of draft CDD
- Marketed overseas with record of stability
- Ideal after day 5 post-onset of symptoms

SD NS-1 Cassette RDT’s developed recently
- Available NS-1 RDT’s comprehensively evaluated
- Meets KSA and Attributes of draft CDD
- Required for early diagnosis of dengue between day 0-7 post-onset of symptoms

Together, the two RDT’s can enable dengue diagnosis through all stages of infection to fulfill capability gap.

Slide courtesy of Dr. Subhamoy Pal
Advantages and limitations of different dengue diagnostic tests

<table>
<thead>
<tr>
<th>Diagnostic tests</th>
<th>Advantages</th>
<th>limitations</th>
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</thead>
<tbody>
<tr>
<td>Viral isolation and identification</td>
<td>• Confirmed infection</td>
<td>• Requires acute sample (0–5 days post onset)</td>
</tr>
<tr>
<td></td>
<td>• Specific</td>
<td>• Requires expertise and appropriate facilities</td>
</tr>
<tr>
<td></td>
<td>• Identifies serotypes</td>
<td>• Takes more than 1 week</td>
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<tr>
<td></td>
<td></td>
<td>• Does not differentiate between primary and secondary infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Expensive</td>
</tr>
<tr>
<td>RNA detection</td>
<td>• Confirmed infection</td>
<td>• Potential false-positives owing to contamination</td>
</tr>
<tr>
<td></td>
<td>• Sensitive and specific</td>
<td>• Requires acute sample (0–5 days post onset)</td>
</tr>
<tr>
<td></td>
<td>• Identifies serotype and genotype</td>
<td>• Requires expertise and expensive laboratory equipment</td>
</tr>
<tr>
<td></td>
<td>• Results in 24–48 hours</td>
<td>• Does not differentiate between primary and secondary infection</td>
</tr>
</tbody>
</table>
Advantages and limitations of different dengue diagnostic tests: Serology

<table>
<thead>
<tr>
<th>Diagnostic Tests</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM or IgG seroconversion</td>
<td>• Confirmed infection</td>
<td>• IgM levels can be low in secondary infections</td>
</tr>
<tr>
<td></td>
<td>• Least expensive</td>
<td>• Confirmation requires two or more serum samples</td>
</tr>
<tr>
<td></td>
<td>• Easy to perform</td>
<td>• Can differentiate between primary and secondary infection*</td>
</tr>
<tr>
<td>IgM detection (single sample)</td>
<td>• Identifies probable dengue cases</td>
<td>• IgM levels can be low in secondary infections</td>
</tr>
<tr>
<td></td>
<td>• Useful for surveillance, tracking outbreaks and monitoring effectiveness of interventions</td>
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</tr>
</tbody>
</table>

*Primary infection: IgM-positive and IgG-negative (if samples are taken before day 8–10); secondary infection: IgG should be higher than 1,280 haemagglutination inhibition in convalescent serum.
# Advantages and limitations of different dengue diagnostic tests: Antigen Detection

<table>
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<tr>
<th>Diagnostic Test</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
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</table>
| Clinical specimens (for example, using blood in an NS1 assay) | • Confirmed infection  
• Easy to perform  
• Less expensive than virus isolation or RNA detection | • Not as sensitive as virus isolation or RNA detection |
| Tissues from fatal cases (for immunohistochemistry, for example) | • Confirmed infection | • Not as sensitive as virus isolation or RNA detection  
• Requires expertise in pathology |
Tourniquet Test (TT)

- Positive in up to 50% of patients with classical dengue and almost all with DHF
- Non-specific

- Procedure:
  - Inflate BP cuff halfway between systolic and diastolic BP for 5 minutes
  - Release
  - Count # petechiae in a quarter-sized patch below the cuff
  - >20 is positive
Sample Prep

- Collect 2 separate red gel separator tubes (“tiger-tops”)
  - Gently invert 5 times
  - Allow blood to clot min. 30 min (vertical)
  - Centrifuge at full speed (1100-1399 G) for 10 min
  - Pipette off serum into separate cryovials
  - Refrigerate or ice bath (2-8°C, ELISA/PRNT)
  - RT-PCR: store @ 2-8°C for up to 6 hours (immediate RNA extraction possible) otherwise, store @ -20°C for up to 14 days. Limit to one freeze-thaw cycle.
  - Isolation: store @ -80°C until ready for transport
Rx

- Symptomatic, supportive
  - CAREFUL fluid management
- Acetaminophen
  - NO Aspirin (Reye's)
- Oral Fluid Replacement
  - If can take PO, no complications, mentally with it
- Serial monitoring of (to trigger IV therapy)
  - HR, BP, Skin perfusion, Urine output, Hct (>20%)
- Development of any warning sign
  - Hospitalization, close observation
  - Judicious use of IV fluids on poor PO, rapidly increasing Hct.
Rx (2)

- Shock
  - PROMPT fluid resuscitation
  - Isotonic crystalloid (initially)/colloid (for those presenting in profound shock, no response to crystalloid) solutions
  - Keep to minimum required to support CV stability
  - Plasma, cryoprecipitates, whole blood (care to not fluid overload)
  - PREVENTIVE transfusions should be avoided
- Desmopressin? IV gamma globulin? Steroids? Drugs (chloroquine, balapiravir, statins)? No evidence for efficacy
- Beware pulmonary edema: may need PPV
  - DHF-DSS is the 3\textsuperscript{rd} most common cause of ARDS in hospitalized children in Malaysia
Dengue Prevention

• Prevention:
  – There is no prophylactic drug for dengue
  – There is no licensed vaccine (yet) to prevent dengue
  – Reduce risk by use of personal protective measures (DEET, permethrin-treated uniforms, screened windows, mosquito netting) and local vector control (eliminate breeding sites, insecticides)
  – New approaches to vector control
    • Genetically altered male mosquitoes
    • Embryonic introduction of wolbachia into A. aegypti
Prevention

- Mantenga limpio el patio de su casa.
- Coloque hacia abajo recipientes.
- Tape depósitos de agua.
- Tire latas, botellas, neumáticos y otros objetos en desuso que acumulen agua.
- Renueve periodicamente el agua de floreros, peceras, bebederos de mascotas, etc.

Entre todos podemos prevenirlo

Todas contra el Dengue
Eliminemos los criaderos

http://wahootours.hyperboards.com/action/view_topic/topic_id/5675
And now for something completely different…

• 31 y/o female recently returned from Singapore…

• Fever (39.5°C), nausea, myalgias, back pain, HA, bilateral conjunctivitis, severe bilateral arthralgias (shoulders, knees, ankles, elbows, wrists, fingers).

• Lab: Lymphopenia (0.6 G/L), AST 177 UI/L, ALT 116 UI/L, LDH 780 UI/L, Nl Bili, CRP 64 mg/L.

• Course: developed chronic distal arthritis and tenosynovitis, swelling of the joints without fluid accumulation.
Chikungunya

Chikungunya is a rare viral fever caused by an Alpha Virus. It is spread by the bite of an infected mosquito, Aedes aegypti.
Summary

• Dengue is a significant threat to the US military and civilian populations in endemic areas.
  – Recognize atypical presentations: maintain healthy suspicion
  – May not have high case fatality rates, but illness will significantly affect mission(s)

• Vaccine development is underway and is challenging
  – WRAIR is a leader in developing dengue vaccines
  – Several candidate vaccines are in the pipeline
Dengue and Hemorrhagic Fever
A Potential Threat to Public Health in the United States

“Most individuals in the United States are as little concerned about dengue fever as they were a decade ago about West Nile fever. That situation could change if dengue continues its expansion as one of the world’s most aggressive reemerging infections.”

Viral Disease Branch

Walter Reed Army Institute of Research
Division of Viral Diseases