STATE OF MARYLAND
DHMH
Maryland Department of Health and Mental Hygiene
201 W. Preston Street • Baltimore, Maryland 21201
Martin O'Malley, Governor – Anthony G. Brown, Lt. Governor – Joshua M. Sharfstein, M.D. Secretary

Laboratories Administration
Robert A. Myers, Ph.D., Director

DATE: August 1, 2014

TO: Medical Laboratory Directors, Local Health Officers, Health Care Providers

FROM: Robert A. Myers, Ph.D. RAM
Director, Laboratories Administration

Maria Paz Carlos, Ph.D. mc
Chief, Division of Virology and Immunology, Laboratories Administration

RE: Availability of Testing for Suspected Travel-Associated Chikungunya and Dengue Virus Infections and Instructions for Submission of Specimens

We want to make you aware of an additional option available for Chikungunya and Dengue testing in Maryland. In response to the emergence of Chikungunya Virus in the Americas including recent locally acquired cases in Florida and the occurrence of travel-associated Dengue cases, the Maryland DHMH Public Health Laboratory is now providing both the real-time PCR and IgM ELISA tests for detecting Chikungunya and Dengue viral infections. These tests are being performed for public health surveillance purposes to identify acute infections from travelers who have recently returned to Maryland from regions where local transmissions have been documented, especially in the Caribbean. Both viruses co-circulate in the same areas, are transmitted by the same mosquitos, and can be difficult to distinguish clinically. Also, co-infections can occasionally occur. A detailed summary of clinical features of Dengue and Chikungunya viral infections can be found at the following CDC website information pages (http://www.cdc.gov/dengue/resources/Dengue&DHF%20Information%20for%20Health%20Care%20Practitioners_2009.pdf and http://www.cdc.gov/chikungunya/hc/index.html).

Clinicians who are considering Dengue and/or Chikungunya viral infections can submit specimens for testing by the MD DHMH Laboratory if the following listed conditions below are met: (**Note:** Specimens submitted without documentation of these criteria will not be accepted for testing).

1) compatible clinical presentation;
2) recent travel to a region where local transmission of either virus has been documented; and
3) acute illness onset date that is compatible with the travel exposures in endemic areas (illness on-set date < 14 days after exposure).

Whole blood (red-top tube/serum separator tube) or sera are acceptable specimens for both real-time PCR and IgM ELISA testing. PCR testing will be performed on acceptable acute phase specimens collected within 8 days of the onset of illness. IgM testing will be performed on specimens collected > 4 days after the on-set of illness. An additional convalescent specimen for IgM testing could be required to rule-out these infections if the acute phase specimen is negative by **both** PCR and IgM testing. For detailed instructions for collecting and submitting acceptable specimens, please refer to the attached instructions on the DHMH Serological Testing Request Form No. 4677 Sample Form (Travel-Associated Chikungunya and Dengue Viral Infections Instructions for Specimen Submission)
or go to the MD DHMH Laboratories website (http://dhmh.maryland.gov/laboratories).

Clinical laboratories that are currently performing Chikungunya and Dengue virus testing should be aware that reporting cases of infections and submitting clinical materials (i.e. serum, CSF) from these cases to the MD DHMH Laboratories is required by statute (Annotated Code of Maryland Health-General Article, §§18-201, 18-202, and 18-205, and Code of Maryland Regulations 10.06.01.03C: #9 Arboviral Infections). The DHMH Serological Testing Request Form No. 4677 should be used to submit these specimens to the MD DHMH Laboratories.

The MD DHMH Laboratories can be contacted for Chikungunya, Dengue Fever and other Arboviral testing at (410)767-6153 or (410)767-5819 during normal business hours from 8:00 a.m. - 4:30 p.m. Monday through Friday.

Enc:
DHMH Laboratories Serological Testing Sample Form (Travel-Associated Chikungunya and Dengue Viral Infections Instructions for Specimen Submission)
CDC Chikungunya Fact Sheet: General Information for Healthcare Providers
CDC Chikungunya Fact Sheet: Clinical Management in Dengue-Endemic Areas
CDC Dengue and Dengue Hemorrhagic Fever Information for Health Care Providers

cc: Dr. Laura Herrera
Dr. David Blythe
Dr. Lucy Wilson
Dr. Katherine Feldman

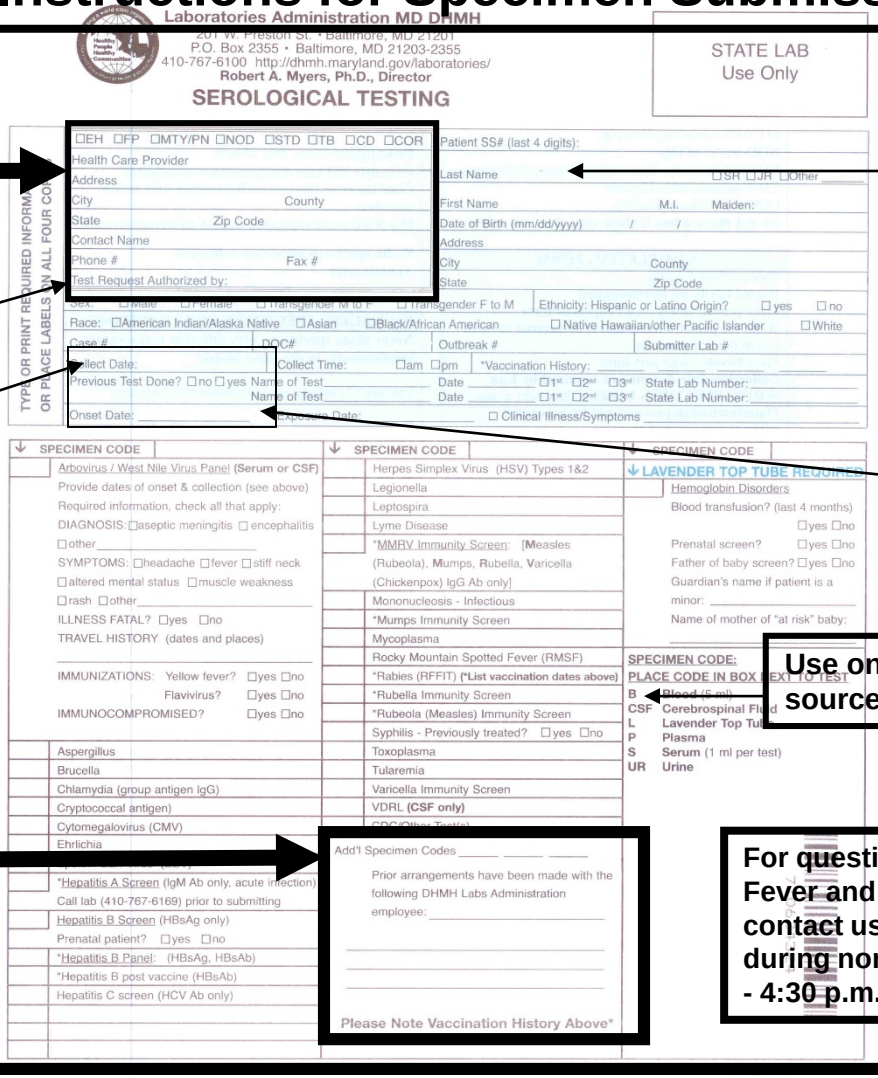
P.O. BOX 2355 • Baltimore, Maryland 21203-2355
410-767-6100 • TTY for Disabled - Maryland Relay Service 1-800-735-2258
Toll Free 1-877-4MD-DHMH • *Web Site:* www.dhmh.state.md.us/labs/

Travel-Associated Chikungunya and Dengue Viral Infections
Instructions for Specimen Submission

Must complete submitter information and include the name of the authorized person requesting the test.

Fill in the date specimen was collected.

Write Chikungunya or Dengue Fever IgM EIA and PCR.
Indicate Blood/Serum.
Complete patient's travel history, symptoms, and vaccination history.



Laboratories Administration MD DHMH
201 W. Preston St. • Baltimore, MD 21201
P.O. Box 2355 • Baltimore, MD 21203-2355
410-767-6100 http://dhmh.maryland.gov/laboratories/
Robert A. Myers, Ph.D., Director

SEROLOGICAL TESTING

STATE LAB Use Only

TYPE OR PRINT REQUIRED INFORMATION OR PLACE LABELS ON ALL FOUR CORNERS

☐EH ☐FP ☐MTY/PN ☐NOD ☐STD ☐TB ☐CD ☐COR
Health Care Provider
Address
City County
State Zip Code
Contact Name
Phone # Fax #
Test Request Authorized by:

Patient SS# (last 4 digits):
Last Name ☐SR ☐JR ☐Other
First Name M.I. Maiden:
Date of Birth (mm/dd/yyyy) / /
Address
City County
State Zip Code
Sex: ☐Male ☐Female ☐Transgender M to F ☐Transgender F to M Ethnicity: Hispanic or Latino Origin? ☐yes ☐no
Race: ☐American Indian/Alaska Native ☐Asian ☐Black/African American ☐Native Hawaiian/other Pacific Islander ☐White
Case # DOC# Outbreak # Submitter Lab #
Collect Date: Collect Time: ☐am ☐pm *Vaccination History:
Previous Test Done? ☐no ☐yes Name of Test Date ☐1st ☐2nd ☐3rd State Lab Number:
Name of Test Date ☐1st ☐2nd ☐3rd State Lab Number:
Onset Date: Exposure Date: ☐Clinical Illness/Symptoms

SPECIMEN CODE
Arbovirus / West Nile Virus Panel (Serum or CSF)
Provide dates of onset & collection (see above)
Required information, check all that apply:
DIAGNOSIS: ☐aseptic meningitis ☐encephalitis ☐other
SYMPTOMS: ☐headache ☐fever ☐stiff neck ☐altered mental status ☐muscle weakness ☐rash ☐other
ILLNESS FATAL? ☐yes ☐no
TRAVEL HISTORY (dates and places)
IMMUNIZATIONS: Yellow fever? ☐yes ☐no Flavivirus? ☐yes ☐no
IMMUNOCOMPROMISED? ☐yes ☐no

Aspergillus
Brucella
Chlamydia (group antigen IgG)
Cryptococcal antigen)
Cytomegalovirus (CMV)
Ehrlichia
*Hepatitis A Screen (IgM Ab only, acute infection)
Call lab (410-767-6169) prior to submitting
Hepatitis B Screen (HBsAg only)
Prenatal patient? ☐yes ☐no
*Hepatitis B Panel: (HBsAg, HBsAb)
*Hepatitis B post vaccine (HBsAb)
Hepatitis C screen (HCV Ab only)

SPECIMEN CODE
Herpes Simplex Virus (HSV) Types 1&2
Legionella
Leptospira
Lyme Disease
*MMRV Immunity Screen: [Measles (Rubeola), Mumps, Rubella, Varicella (Chickenpox) IgG Ab only]
Mononucleosis - Infectious
*Mumps Immunity Screen
Mycoplasma
Rocky Mountain Spotted Fever (RMSF)
*Rabies (RFFIT) (*List vaccination dates above)
*Rubella Immunity Screen
*Rubeola (Measles) Immunity Screen
Syphilis - Previously treated? ☐yes ☐no
Toxoplasma
Tularemia
Varicella Immunity Screen
VDRL (CSF only)
CDC/Other Toxins

Add'l Specimen Codes
Prior arrangements have been made with the following DHMH Labs Administration employee:
Please Note Vaccination History Above*

SPECIMEN CODE
↓ LAVENDER TOP TUBE REQUIRED
Hemoglobin Disorders
Blood transfusion? (last 4 months) ☐yes ☐no
Prenatal screen? ☐yes ☐no
Father of baby screen? ☐yes ☐no
Guardian's name if patient is a minor:
Name of mother of "at risk" baby:

SPECIMEN CODE:
PLACE CODE IN BOX NEXT TO TEST
B Blood (5 ml)
CSF Cerebrospinal Fluid
L Lavender Top Tube
P Plasma
S Serum (1 ml per test)
UR Urine

Patient's first and last names must be on the specimen container and match exactly to the lab slip.

Onset date field **must** be completed. Onset date of patient's symptoms is required for test results interpretation.

Use only these codes to provide the source of the specimen.

For questions on Chikungunya, Dengue Fever and other Arboviral testing, please contact us at (410)767-6153 or (410)767-5819 during normal business hours from 8:00 a.m. - 4:30 p.m. Monday through Friday.

Whole blood (red-top tube/serum separator tube) or sera are acceptable specimens for both real-time PCR and IgM ELISA testing.
For blood collection, the following blood tubes listed below should only be used. Heparin (green top) and EDTA (purple top) are NOT ACCEPTABLE.
1. Serum Separator Tube (SST – tiger/speckled top). The best type of tube is serum separator. Collect 6 mls of blood. The blood should be allowed to coagulate and tubes should be spun to separate the serum from the clot prior to shipping.
2. Red Top Tube (no additive). Collect 6 mls of blood. The blood must be allowed to coagulate, the tube centrifuged, and the serum drawn off into a clean tube prior to shipping. Please submit at least 3 ml. of serum.
Go to the DHMH Laboratory website for further information: www.dhmh.maryland.gov/laboratories

CHIKUNGUNYA

Information for healthcare providers

Background

- Mosquito-borne viral disease characterized by acute onset of fever and severe polyarthralgia
- Often occurs as large outbreaks with high attack rates
- Outbreaks have occurred in countries in Africa, Asia, Europe, and the Indian and Pacific Oceans
- In late 2013, first local transmission in the Americas was reported on islands in the Caribbean

Chikungunya virus

- Single-stranded RNA virus
- Genus *Alphavirus*; Family *Togaviridae*

Mosquito vectors

- *Aedes aegypti* and *Aedes albopictus* are the primary vectors (above)
- Both are aggressive daytime biting mosquitoes

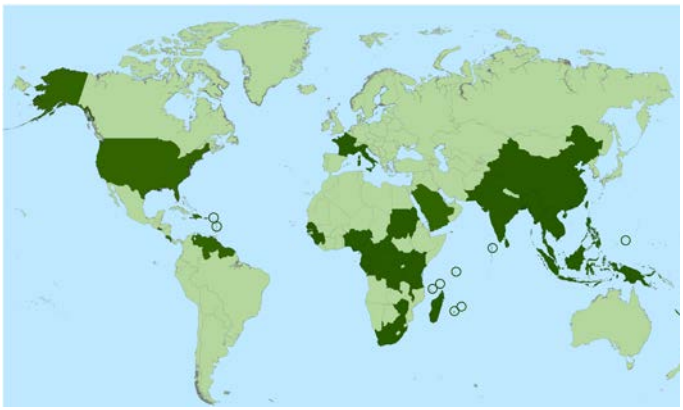
Animal hosts

- Humans are the primary host of chikungunya virus during epidemic periods

Clinical findings

- Majority of infected people become symptomatic
- Incubation period usually 3–7 days (range 1–12 days)
- Acute onset of fever and polyarthralgia are the primary clinical findings
- Joint symptoms usually symmetric and often occur in hands and feet; they can be severe and debilitating
- Other symptoms: Headache, myalgia, arthritis, conjunctivitis, nausea/vomiting, maculopapular rash
- Lymphopenia, thrombocytopenia, elevated creatinine, and elevated hepatic transaminases are the most common clinical laboratory findings

Countries with reported local transmission of chikungunya virus (as of July 2014)



Laboratory testing

- Evaluate serum or plasma by:
  - Viral culture to detect virus in first 3 days of illness
  - RT-PCR to detect viral RNA in first 8 days of illness
  - Serology to detect IgM, IgG, and neutralizing antibodies that develop toward the end of the first week of illness (≥4 days post illness onset)
- Chikungunya testing is performed at CDC, several state health departments, and one commercial laboratory
- Contact your state health department for more information and to facilitate testing

Clinical course and outcomes

- Acute symptoms typically resolve within 7–10 days
- Rare complications include uveitis, retinitis, myocarditis, hepatitis, nephritis, bullous skin lesions, hemorrhage, meningoencephalitis, myelitis, Guillain-Barré syndrome, and cranial nerve palsies
- Persons at risk for severe disease include neonates exposed intrapartum, older adults (e.g., > 65 years), and persons with underlying medical conditions (e.g., hypertension, diabetes, or cardiovascular disease)
- Some patients might have relapse of rheumatologic symptoms (e.g., polyarthralgia, polyarthritis, tenosynovitis) in the months following acute illness
- Studies report variable proportions of patients with persistent joint pains for months to years

Chikungunya and dengue

- Difficult to distinguish chikungunya and dengue based on clinical findings alone
- Chikungunya and dengue viruses are transmitted by the same mosquitoes
- The viruses can circulate in the same area and cause occasional co-infections in the same patient
- Chikungunya virus more likely to cause high fever, severe polyarthralgia, arthritis, rash, and lymphopenia
- Dengue virus more likely to cause neutropenia, thrombocytopenia, hemorrhage, shock, and deaths
- Patients with suspected chikungunya should be managed as dengue until dengue has been ruled out
  - Proper clinical management of dengue reduces the risk of medical complications and death
  - Aspirin and other NSAIDs can increase the risk of hemorrhage in patients with dengue

Treatment and clinical management

- No specific antiviral therapy; treatment is symptomatic
- Assess hydration and hemodynamic status and provide supportive care as needed
- Evaluate for other serious conditions (e.g., dengue, malaria, and bacterial infections) and treat or manage appropriately
- Collect specimens for diagnostic testing
- Use acetaminophen or paracetamol for initial fever and pain control
  - If inadequate, consider using narcotics or NSAIDs
  - If the patient may have dengue, do not use aspirin or other NSAIDs (e.g., ibuprofen, naproxen, toradol) until they have been afebrile ≥48 hours and have no warning signs for severe dengue*
- Persistent joint pain may benefit from use of NSAIDs, corticosteroids, or physiotherapy

*Warning signs for severe dengue include severe abdominal pain, persistent vomiting, mucosal bleeding, pleural effusion or ascites, lethargy, enlarged liver, and increased hematocrit with decrease in platelet count

Differential diagnosis

- Depends on residence, travel history, and exposures
- Consider dengue, leptospirosis, malaria, rickettsia, group A streptococcus, rubella, measles, parvovirus, enteroviruses, adenovirus, other alphavirus infections (e.g., Mayaro, Ross River, Barmah Forest, O'nyong-nyong, and Sindbis viruses), post-infections arthritis, and rheumatologic conditions

Surveillance and reporting

- Chikungunya virus infection should be considered in patients with acute onset of fever and polyarthralgia, especially travelers who recently returned from areas with known virus transmission
- Healthcare providers are encouraged to report suspected chikungunya cases to their state or local health department to facilitate diagnosis and mitigate the risk of local transmission
- Health departments should perform surveillance for chikungunya cases in returning travelers and be aware of the risk of possible local transmission in areas where *Aedes* species mosquitoes are active
- State health departments are encouraged to report confirmed chikungunya virus infections to CDC

Prevention and control

- No vaccine or medication is available to prevent chikungunya virus infection or disease
- Reduce mosquito exposure
  - Use air conditioning or window/door screens
  - Use mosquito repellents on exposed skin
  - Wear long-sleeved shirts and long pants
  - Wear permethrin-treated clothing
  - Empty standing water from outdoor containers
  - Support local vector control programs
- People suspected to have chikungunya or dengue should be protected from further mosquito exposure during the first week of illness to reduce the risk of further transmission
- People at increased risk for severe disease should consider not traveling to areas with ongoing chikungunya outbreaks

FOR MORE INFORMATION VISIT: www.cdc.gov/chikungunya/

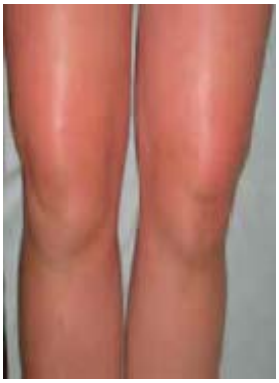
Revised July 22, 2014

CHIKUNGUNYA

Clinical management in dengue-endemic areas

Clinical findings

- Acute onset of fever and polyarthralgia are the primary clinical findings
- Joint symptoms usually symmetric and often occur in hands and feet
- Other symptoms: Headache, myalgia, arthritis, conjunctivitis, nausea/vomiting, or maculopapular rash
- Lymphopenia, thrombocytopenia, elevated creatinine, and elevated hepatic transaminases are the most common clinical laboratory findings
- Mortality rare but joint symptoms can be severe and debilitating

		
Edematous polyarthritis of the hands	Periarticular swelling and joint effusion in knees	Maculopapular rash in extremities, including palms

Chikungunya and dengue

- Difficult to distinguish chikungunya and dengue based on clinical findings alone
- Chikungunya and dengue viruses transmitted by the same mosquitoes
- The viruses can circulate in the same area and cause occasional co-infections in the same patient
- Chikungunya virus more likely to cause high fever, severe polyarthralgia, arthritis, rash, and lymphopenia
- Dengue virus more likely to cause neutropenia, thrombocytopenia, hemorrhage, shock, and death
- **Patients with suspected chikungunya should be managed as dengue until dengue has been ruled out**
    - Proper clinical management of dengue reduces the risk of medical complications and death
    - Aspirin and other NSAIDs can increase the risk of hemorrhage in patients with dengue

Clinical and laboratory features of chikungunya virus infections compared with dengue virus infections

	Chikungunya	Dengue
Fever (>39°C)	+++	++
Arthralgia	+++	+/-
Arthritis	+	-
Headache	++	++
Rash	++	+
Myalgia	+	++
Hemorrhage	+/-	++
Shock	-	+
Lymphopenia	+++	++
Neutropenia	+	+++
Thrombocytopenia	+	+++
Hemoconcentration	-	++

Treatment and clinical management

- No specific antiviral therapy; treatment is symptomatic
- Assess hydration and hemodynamic status and provide supportive care as needed
- Evaluate for other serious conditions (e.g., dengue, malaria, and bacterial infections) and treat or manage appropriately
- Collect specimens for diagnostic testing
- Use acetaminophen or paracetamol for initial fever and pain control
    - If inadequate, consider using narcotics or NSAIDs
    - **If the patient may have dengue, do not use aspirin or other NSAIDs (e.g., ibuprofen, naproxen, toradol) until they have been afebrile ≥48 hours and have no warning signs for severe dengue***
- Persistent joint pain may benefit from use of NSAIDs, corticosteroids, or physiotherapy

---

*Warning signs for severe dengue include severe abdominal pain, persistent vomiting, mucosal bleeding, pleural effusion or ascites, lethargy, enlarged liver, and increased hematocrit with decrease in platelet count

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention

Dengue and Dengue Hemorrhagic Fever

Information for Health Care Practitioners

Dengue is a mosquito-borne disease caused by any one of four closely related dengue viruses (DENV-1, -2, -3, and -4). Infection with one serotype of DENV provides immunity to that serotype for life, but provides no long-term immunity to other serotypes. Thus, a person can be infected as many as four times, once with each serotype. Dengue viruses are transmitted from person to person by *Aedes* mosquitoes (most often *Aedes aegypti*) in the domestic environment. Epidemics have occurred periodically in the Western Hemisphere for more than 200 years. In the past 30 years, dengue transmission and the frequency of dengue epidemics have increased greatly in most tropical countries in the American region.

Clinical Diagnosis

Dengue

Classic dengue fever, or "break bone fever," is characterized by acute onset of high fever 3–14 days after the bite of an infected mosquito. Symptoms include frontal headache, retro-orbital pain, myalgias, arthralgias, hemorrhagic manifestations, rash, and low white blood cell count. The patient also may complain of anorexia and nausea. Acute symptoms, when present, usually last about 1 week, but weakness, malaise, and anorexia may persist for several weeks. A high proportion of dengue infections produce no symptoms or minimal symptoms, especially in children and those with no previous history of having a dengue infection.

The main medical complications of classic dengue fever are febrile seizures and dehydration. Treatment of dengue fever emphasizes

- Relieving symptoms of pain.
- Controlling fever.
- Telling patients to avoid aspirin and other nonsteroidal, anti-inflammatory medications because they may increase the risk for hemorrhage.
- Reminding patients to drink more fluids, especially when they have a high fever.

Dengue Hemorrhagic Fever and Dengue Shock Syndrome

Some patients with dengue fever go on to develop dengue hemorrhagic fever (DHF), a severe and sometimes fatal form of the disease. Around the time the fever begins to subside (usually 3–7 days after symptom onset), the patient may develop warning signs of severe disease. Warning signs include severe abdominal pain, persistent vomiting, marked change in temperature (from fever to hypothermia), hemorrhagic manifestations, or change in mental status (irritability, confusion, or obtundation). The patient also may have early signs of shock, including restlessness, cold clammy skin, rapid weak pulse, and narrowing of the pulse pressure (systolic blood pressure – diastolic blood pressure). Patients with dengue fever should be told to return to the hospital if they develop any of these signs.

DHF is currently defined by the following four World Health Organization (WHO) criteria:

- Fever or recent history of fever lasting 2–7 days.
- Any hemorrhagic manifestation.
- Thrombocytopenia (platelet count of <100,000/mm3).
- Evidence of increased vascular permeability.

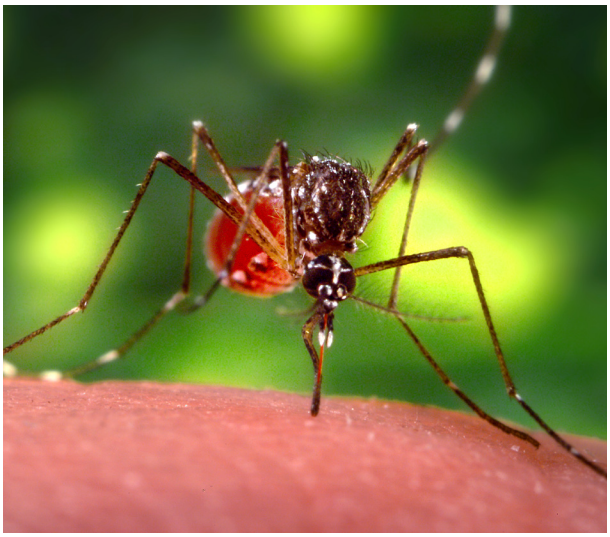
The most common hemorrhagic manifestations are mild and include a positive tourniquet test, skin hemorrhages (petechiae, hematomas), epistaxis (nose bleed), gingival bleeding (gum bleed), and microscopic hematuria. More serious types of hemorrhage include vaginal bleeding, hematemesis, melena, and intracranial bleeding.

Evidence of plasma leakage due to increased vascular permeability consists of at least one of the following:

- An elevated hematocrit ≥20% above the population mean hematocrit for age and sex.
- A decline in hematocrit after volume-replacement treatment of ≥20% of the baseline hematocrit.
- Presence of pleural effusion or ascites detected by radiography or other imaging method.
- Hypoproteinemia or hypoalbuminemia as determined by laboratory test.

WHO is currently reevaluating the clinical case definition for dengue fever and DHF. Studies from different countries have reported life-threatening complications from dengue in the absence of one or more of the current criteria for DHF. Despite the name, the critical feature that distinguishes DHF from dengue fever is not hemorrhaging, but rather plasma leakage resulting from increased vascular permeability.

Dengue shock syndrome (DSS) is defined as any case that meets the four criteria for DHF and has evidence of circulatory failure manifested by



A female Aedes aegypti *mosquito obtaining a blood-meal from a human host through her proboscis, which penetrates the host's skin.*

(1) rapid, weak pulse and narrow pulse pressure (≤20 mmHg [2.7 kPa]) or (2) hypotension for age, restlessness, and cold, clammy skin. Patients with dengue can rapidly progress into DSS, which, if not treated correctly, can lead to severe complications and death.

Fatality rates among patients with DSS can be 10% or higher but, with early recognition and treatment, can be less than 1%. DHF and DSS can occur in both children and adults.

What to Look for When You Evaluate Patients for DHF

EVALUATE the patient's heart rate, capillary refill, skin color and temperature, peripheral pulse volume, pulse pressure, and blood pressure. A drop in systolic blood pressure is usually the last sign and appears only when the patient is in shock.

LOOK FOR evidence of bleeding on the skin and at other sites.

LOOK FOR evidence of increased capillary permeability (e.g., pleural effusions, ascites, hemoconcentration).

MEASURE and ask about urine output.

How to Treat Dengue Fever

- Tell patients to drink plenty of fluids and get plenty of rest.
- Tell patients to take antipyretics to control their temperature. Children with dengue are at risk for febrile seizures during the febrile phase of illness.
- Warn patients to avoid aspirin and other nonsteroidal, anti-inflammatory medications because they increase the risk of hemorrhage.
- Monitor your patients' hydration status during the febrile phase of illness. Educate patients and parents about the signs of dehydration and have them monitor their urine output.
- If patients cannot tolerate fluids orally, they may need IV fluids. Assess hemodynamic status frequently by checking the patient's heart rate, capillary refill, pulse pressure, blood pressure, and urine output.
- Perform hemodynamic assessments, baseline hematocrit testing, and platelet counts.
- **Continue to monitor your patients closely during defervescence. The critical phase of dengue begins with defervescence and lasts 24–48 hours.**

Clinical Management

Even for outpatients, stress the need to maintain adequate hydration. Monitoring for warning signs of severe dengue and initiating early appropriate treatment are key to preventing complications such as prolonged shock and metabolic acidosis. Successful management of DHF and DSS includes judicious and timely IV fluid replacement therapy with isotonic solutions and frequent reassessment of the patient's hemodynamic status and vital signs during the critical phase. Health care providers should learn to recognize this disease at an early stage. To manage pain and fever, patients should be given acetaminophen. Aspirin and nonsteroidal, anti-inflammatory medications may aggravate the bleeding tendency associated with some dengue infections and, in children, can be associated with the development of Reyes syndrome.

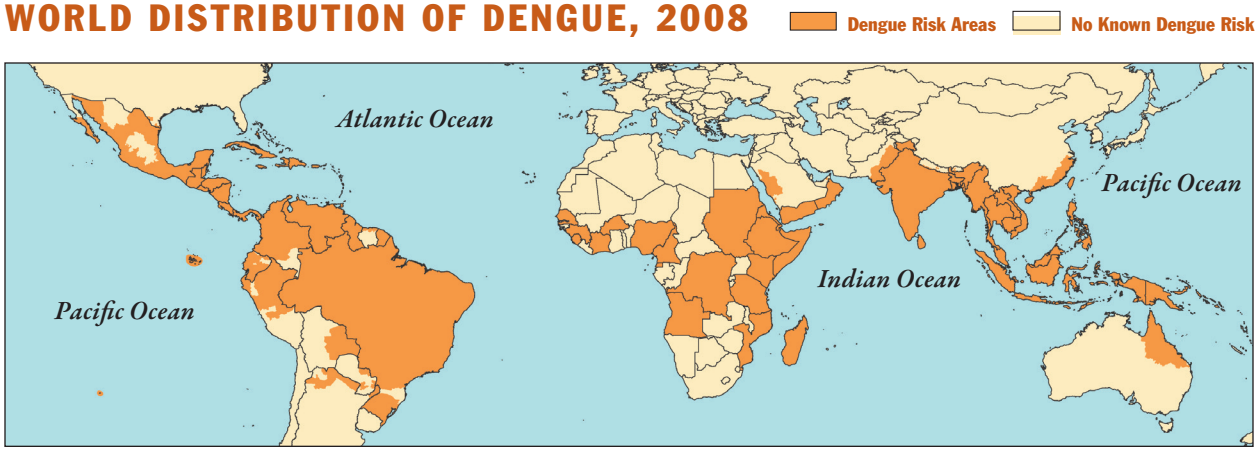
Laboratory Diagnosis

Unequivocal diagnosis of dengue infection requires laboratory confirmation, either by isolating the virus or detecting dengue-specific antibodies. For virus isolation or detection of DENV RNA in serum specimens by serotype-specific, real-time reverse transcriptase polymerase chain reaction (RT-PCR), an acute-phase serum specimen should be collected within 5 days of symptom onset. If the virus cannot be isolated or detected from this sample, a convalescent-phase serum specimen is needed at least 6 days after the onset of symptoms to make a serologic diagnosis by testing for IgM antibodies to dengue with an IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA).

Acute-phase and convalescent-phase serum samples should be sent to the state health department or to the Centers for Disease Control and Prevention (CDC) for testing. Acute-phase samples for virus diagnosis may be stored on dry ice (-70°C) or, if delivery can be made within 1 week, stored unfrozen in a refrigerator (4°C). Convalescent-phase samples should be sent in a rigid container without ice, if next-day delivery is assured. Otherwise, they should be shipped on ice in an insulated container to avoid heat exposure during transit.

Most tests for anti-dengue antibodies yield nonspecific results for flaviviruses, including West Nile and St. Louis encephalitis viruses. Because commercial kits may vary in sensitivity and specificity, test results may need to be confirmed by a reference laboratory.

WORLD DISTRIBUTION OF DENGUE, 2008



Epidemiology

A dengue epidemic requires the presence of

- The vector mosquito (usually *Aedes aegypti*).
- The dengue virus.
- A large number of susceptible human hosts.

Outbreaks may be explosive or progressive, depending on the density and efficiency by which the vector can be infected, the serotype and strain of the dengue virus, the number of susceptible (nonimmune) humans in the population, and the amount of vector-human contact.

Dengue should be considered as the possible etiology when leptospirosis, enterovirus, influenza, rubella, or measles are suspected in a dengue-receptive area (i.e., at a time and place where vector mosquito populations are abundant and active). In Puerto Rico and most countries of the Caribbean Basin, *Aedes aegypti* is abundant year-round. In the continental United States, this species is seasonally abundant in Arizona, Louisiana, southern New Mexico, Texas, Florida, Alabama, Georgia, Mississippi, North and South Carolina, Oklahoma, Kentucky, and Tennessee. Given the competent vectors and susceptible population in the continental United States, isolated dengue outbreaks may occur (the last reported dengue outbreak was in Texas in 2005).

In 1985, a mosquito from Asia, *Aedes albopictus*, was found in the United States. This species is now found in most states in the southeastern part of the United States, as well as in Argentina, Barbados, Bolivia, Brazil, the Cayman Islands, Colombia, Cuba, the Dominican Republic, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, and Paraguay. Although its contact with humans and its density in urban areas are not as great as that of *Aedes aegypti*, this species also can transmit dengue viruses.

As noted previously, the frequency of epidemic disease has increased significantly in the past 30 years. Modern transportation makes it easy for travelers to visit virtually any location on the globe, including areas of the world where dengue is endemic. Although travel-associated dengue and limited outbreaks do occur in the continental United States, most dengue cases in U.S. citizens occur as a result of endemic transmission in some of the U.S. territories. CDC conducts laboratory-based, passive dengue surveillance in Puerto Rico in collaboration with the Puerto Rico Department of Health (PRDH). The PRDH Web site provides a weekly dengue surveillance report produced by CDC and PRDH at http://www.salud.gov.pr.

If a dengue-like illness is observed in a person in the continental United States who has recently traveled to a tropical area, acute and convalescent blood specimens, associated clinical information, and a brief travel history should be sent to the state public health laboratory with a request that the specimens be tested for dengue there or sent to CDC's Dengue Branch in San Juan, Puerto Rico. Contact the CDC Dengue Branch for more information if needed. In Puerto Rico and the U.S. Virgin Islands, specimens and clinical information can be sent through the respective department of health or directly to the CDC Dengue Branch in San Juan.

For further information, contact
Dengue Branch, Centers for Disease Control and Prevention; 1324 Cañada Street; San Juan, Puerto Rico 00920-3860; Tel: (787) 706-2399; Fax: (787) 706-2496