DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
National Center for Infectious Diseases
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Criteria for the processing of dengue samples at the CDC Dengue Branch, San Juan, Puerto Rico

The diagnosis and treatment of dengue and dengue hemorrhagic fever are guided by the symptoms and findings that the patient presents, and cannot depend on laboratory confirmation, since routine tests can not confirm dengue with the speed required for patients in critical condition. The processing of samples for serologic diagnosis takes approximately one week, and the PCR results take approximately 3 days. Even so, it is necessary to eventually have a confirmation of the diagnosis, to exclude other etiologic possibilities, and to guide the follow-up of the patient's convalescence. The CDC Dengue Branch provides dengue testing free of cost to submitting physicians, state and private laboratories.

To obtain correct data on the spread of disease, adequately decide what laboratory tests to use, correctly interpret test results, and to assure that results get to the person who requested them, the following information is indispensable:

- Complete name, age and sex of the patient
- Home address
- Date of onset of symptoms
- Date that sample was obtained
- Complete name and mailing address of the physician, laboratory, clinic or hospital that the result should be sent to.

Samples without the above-mentioned information, or written with illegible handwriting or with more than a month from date of sample collection to date of arrival at CDC, will not be analyzed.

The clinical samples that are processed weekly are of great importance to determine the serotype and genetic make-up of viruses being transmitted in the region, and to determine their geographic distribution. This information can be used to detect risk factors that may lead to a

new and more severe pattern of disease, and to refine the clinical diagnostic ability of attending physicians. Diagnostic bleeding is not provided to persons referred to CDC.

In case of a severe dengue epidemic, CDC Dengue Branch will promptly analyze samples received with the minimum above-mentioned information. If number of specimens exceeds laboratory capacity, testing may be prioritized in the following order:

- fatal cases,
- cases in intensive care,
- hospitalized cases (with thrombocytopenia, hemorrhage, shock or hemoconcentration),
- all other cases.

We want to emphasize that to maintain efficient dengue surveillance, we will continue processing samples from outpatients and mildly ill persons any time of the year, and especially when dengue incidence in the island is relatively low (usually during the months from April to July).

Case Notification and Shipment of Samples of Suspected Dengue Cases

Dengue is characterized by an acute febrile picture accompanied by headache, retroorbital pain, body pain, often a rash, and other variable symptoms that can include obvious or mild hemorrhagic manifestations (such as a petechial rash) or hemoconcentration, shock or coma. This disease should be considered whenever there is an increase in the number of persons who go to the physician with an acute febrile illness, complaints of "monga" or "influenza," or an increase in the number of clinically diagnosed cases of German measles or regular measles.

Instructions for obtaining and handling samples:

1. Once there is a clinical diagnosis of suspected dengue, take a blood sample (see #4 and #5) and fill out the Dengue Case Investigation Form (see copy attached). **With this Form you comply with the legal reporting requirement.** These forms can be obtained from the CDC Dengue Branch, the CDC Website http://www.cdc.gov/ncidod/dvbid/dengue/resources/DEN%20CASE%20Form%20Eng%202004.pdf and also from the Regional Environmental Health Office of the Puerto Rico Department of Health. They can also be photocopied without restriction.

2. It is of great importance to fill out the Dengue Case Investigation Form in a clear and complete manner. The information received on each case (especially the date of onset of symptoms and date of sample collection) is crucial to select and interpret the laboratory analyses. Furthermore, a complete address makes it possible to identify the area where control measures should be implemented. **Samples without the above-mentioned information, or written in illegible handwriting or with more than a month from date of collection to date of arrival at CDC, will not be analyzed.**

3. The blood sample is taken in a red-top tube (preferably, but if not, you can use a green-top tube). Violet-top tubes (with heparin) should not be used. If dry ice is not available we recommend that after separating the serum, it must be maintained on ice or in a refrigerator until it is delivered to the CDC Dengue Branch. For samples from the USA or the exterior, we recommend to freeze the serum immediately after separated and to send in dry ice. The case investigation forms and the acute blood sample should reach CDC Dengue Branch as soon as possible. They can be sent through the local Environmental Health office. The acute sample can be sent immediately; there is no need to wait until the convalescent sample is taken.

4. To diagnose dengue, the laboratory requires a blood sample taken during the acute period of the disease and a second sample that can be taken from day 6 after the onset of symptoms. Informing the patient about the importance of coming back for a second sample, and giving an appointment for a specific day and hour, will increase the probability of obtaining the second sample. If the patient makes the first visit to the physician on or after day 6 after onset of the symptoms, that sample is enough. In that case, it is not necessary that the patient come for a second sample.

5. Acute-phase samples (taken on or before day 5 after onset of symptoms), will be used mainly for PCR analysis in order to detect virus. Convalescent-phase samples (taken on or after day 6 after beginning of symptoms) will be used mainly for detection of IgM anti-dengue antibodies by enzyme-linked immunosorbent assays (ELISA). Differential diagnosis for dengue and WNV

virus is available; but these tests need to be requested according to clinical presentation.

Type of sample	Interval since the onset of symptoms	Type of Analysis
"Acute"	until day 5	PCR
"Convalescent"	6 or more days	Serology

Samples taken on days 4 and 5 of illness are of low yield for isolation as well as serology.

WHENEVER THERE IS A HOSPITALIZED SEVERE CASE, PLEASE INDICATE IT IN THE CASE INVESTIGATION FORM.

6. Reports will be sent to the physician (if the return address has been indicated) with the results of positive, or clearly negative, cases. In cases with negative virus isolation, we will await a convalescent-phase sample before reporting a result.

7. Results will be reported only to the laboratory or the physician who sent the sample (or an authorized secretary).

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CHECK LIST FOR OBTAINING AND SHIPPING DENGUE DIAGNOSTIC SAMPLES

[ ] Sample

Type of sample	Interval since date of onset of symptoms	Type of Analysis
Acute	up to 5 days	PCR
Convalescent	6 or more days	Serology

[ ] **Form -** "Dengue Case Investigation Form"
Can be obtained from the CDC Dengue Branch in San Juan or Internet: http://www.cdc.gov/ncidod/dvbid/dengue/resources/DEN%20CASE%20Form%20Eng%202004.pdf
Please indicate on the sheet if the case is hospitalized. If it is a very severe case, indicate so on the "Comments" section.
Only the samples received with the information requested below, and written in a legible manner, will be analyzed:

- Complete name, age, and sex of patient
- Home address
- Date of onset of symptoms
- Date sample was obtained
- Complete name and mailing address of the physician, laboratory, clinic, or hospital

[ ] **Tube** - Red or green top (not violet).
[ ] **Labeling** - Tube and case form must agree (indicate the same name of the case).
[ ] **Volume** - 2 cc. (ml.) of centrifuged serum or plasma
[ ] **Storage** - On ice or in a refrigerator (not in a freezer) until it is delivered to the CDC Dengue Branch.
[ ] **Time of shipment** - Not to exceed a month after taking the sample
[ ] **Way of shipment** - Check with local Department of Health.

Reasons for **REJECTING** samples:

- Samples without form, form without sample
- Incomplete or illegible form – especially regarding date of onset of symptoms, date of sample collection
- Hemolyzed or frozen sample, or received more than a month after onset of illness

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DENGUE CASE INVESTIGATION REPORT

CDC Dengue Branch and Puerto Rico Department of Health
1324 Calle Cañada, San Juan, P. R. 00920-3860
Tel. (787) 706-2399, Fax (787) 706-2496

Form Approved OMB No. 0920-0009

FOR CDC DENGUE BRANCH USE ONLY

Case number

SAN ID

GCODE

Specimen #	Days post onset (DPO)	Type	Date Received
S1			____/____/____
S2			____/____/____
S3			____/____/____
S4			____/____/____

Please complete all sections

Hospitalized: ☐ No ☐ Yes → Hospital Name:

Fatal: ☐ Yes ☐ No ☐ UNK

Name of Patient: _____
Last Name First Name Middle Name or Initial

Mental Status Changes: ☐ Yes ☐ No ☐ UNK

If patient is a minor, name of father or primary caregiver: _____
Last Name First Name Middle Name or Initial

Home Address

City, Town: Barrio:

Urbanization or sector:

Street: House / Apt. Number:

Premise No.: Box: P.O. Box:

Road No.: Km: Hm: Tel: Other Tel:

Residence is close to: Zip Code:

Work address:

Patient's Basic Information

Date of Birth: Age: _____ months Sex: ☐ M ☐ F

_____/_____/_____ OR _____ years
Day Month Year

Physician who referred the case

Name of Healthcare Provider:

Phone number: Email address:

Send laboratory results to:

Information about the person filling out this form

Name and title: Phone number:

Name and address of employment:

Must have the following information for sample processing

Day Month Year

Date of first symptom: _____/_____/_____

Date specimen taken:

Serum: First sample _____/_____/_____
(Acute = first 5 days of illness – check for virus)

Second sample _____/_____/_____
(Convalescent = more than 5 days after onset – check for antibodies)

Third sample _____/_____/_____

Tissue for fatal cases (specify): _____/_____/_____

Additional Data

1. How long have you lived in this city? _____
2. Country of birth: _____
3. Have you been diagnosed with dengue before? ☐ Yes ☐ No ☐ UNK
4. When diagnosed? _____/_____ ☐ UNK
   Month Year
5. During the 14 days before onset of illness, did you TRAVEL to other cities or countries? ☐ Yes, another country ☐ Yes, another city ☐ No ☐ UNK
6. WHERE did you TRAVEL?

Criteria for DENGUE HEMORRHAGIC FEVER (#1-4) and shock (#5)

	Yes	No	UNK
1. Fever (>38°C)	☐	☐	☐
2. Platelets ≤100,000/mm3	☐	☐	☐

Platelet count: _____

3. Any hemorrhagic manifestation

	Yes	No	UNK
Petechiae	☐	☐	☐
Purpura/Ecchymosis	☐	☐	☐
Vomit with blood	☐	☐	☐
Blood in stool	☐	☐	☐
Nasal bleeding	☐	☐	☐
Bleeding gums	☐	☐	☐
Blood in urine	☐	☐	☐
Vaginal bleeding	☐	☐	☐
Positive urinalysis (over 5 RBC/hpf or positive for blood)	☐	☐	☐

Tourniquet test ☐ Not done ☐ Pos ☐ Neg

4. Evidence of capillary leak

Pleural or abdominal effusion ☐ Yes ☐ No

Lowest hematocrit _____

Highest hematocrit _____

Lowest serum albumin _____

Lowest serum protein _____

5. Lowest blood pressure _____/_____

Lowest pulse pressure (systolic minus diastolic) _____

Lowest white blood cell count _____

Other symptoms	Yes	No	UNK
Headache	☐	☐	☐
Eye pain	☐	☐	☐
Body pain	☐	☐	☐
Joint pain	☐	☐	☐

Symptoms continued	Yes	No	UNK
Rash	☐	☐	☐
Chills	☐	☐	☐
Pallor or cool skin	☐	☐	☐
Nausea or vomiting	☐	☐	☐
Diarrhea	☐	☐	☐
Abdominal pain	☐	☐	☐
Cough	☐	☐	☐
Conjunctivitis (red eyes)	☐	☐	☐
Nasal Congestion	☐	☐	☐
Sore throat	☐	☐	☐
Jaundice	☐	☐	☐
Convulsion or coma	☐	☐	☐
Pregnant?	☐	☐	☐
Got Yellow Fever Vaccine	☐	☐	☐

Year vaccinated _____ ☐

FOR CDC DENGUE BRANCH USE ONLY

Specimen No.

S^1 _____ S^2 _____ S^3 _____

SEROLOGY

LUMINEX (MIA)

S^1			S^2			S^3		
Test Date	Ag	Titer	Test Date	Ag	Titer	Test Date	Ag	Titer

IgG ELISA

S^1				S^2				S^3			
Test Date	Ag	Screen	Titer	Test Date	Ag	Screen	Titer	Test Date	Ag	Screen	Titer

IgM ELISA

S^1			S^2			S^3		
Test Date	Ag	Value	Test Date	Ag	Value	Test Date	Ag	Value

Neutralization

S^1			S^2			S^3		
Test Date	Screen	Titer	Test Date	Screen	Titer	Test Date	Screen	Titer
DENV-1								
DENV-2								
DENV-3								
DENV-4								
WEST NILE								
SLE								
YFV								

Viral Isolation & PCR

S^1				S^2				S^3			
Test Date	ID	Isotech	IDtech	Test Date	ID	Isotech	IDtech	Test Date	ID	Isotech	IDtech

Serology Lab Director Signature: _____

Virology Lab Director Signature: _____ Overall dengue interpretation: _____