



**Pan American
Health
Organization**



Regional Office of the
World Health Organization

**PAHO/WHO Collaborating Centers and
National Reference Laboratories
That Diagnose Dengue**

Pasteur Institute, French Guiana



**INSTITUT PASTEUR
DE LA GUYANE**

Date: January 2007

Name	Position	Institution
Dr Jacques MORVAN Dr Philippe DUSSART	Director Assistant Director	Centre National de Référence des Arbovirus Institut Pasteur de la Guyane

Capacity

1. Please indicate if your reference laboratory is able to carry out the following tests:

Test		Name hot protocol used	Comments
Mac ELISA	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	MAC-ELISA	Talarmin et al, JCM. 1998, adapted from Kuno G et al. Am J Trop Med Hyg. 1987.
ELISA IgG	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	Indirect ELISA	
Hemagglutination Inhibition	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>		
Plate Reduction Neutralization Test (PRNT)	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>		
Viral Isolation	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>		
Typing of isolated viruses	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	Viral typing by IF	
RT-PCR	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	Using Lanciotti primers	Lanciotti et al, JCM. 1992.
PCR in real time	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>		

Detection of antigen by ELISA	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	Using the Platelia Dengue NS1 Antigen test from Bio-Rad Laboratories	One-step sandwich-format microplate enzyme immunoassay for detection of the dengue virus NS1 antigen.
Detection antigen by immunohistochemistry	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>		
Others	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>		

2. How many people are working on the diagnosis and characterization of dengue virus within the laboratory?

5

Techniques

3. What types of specimens are used routinely for the following techniques of diagnostic and isolation of the dengue virus?

Please mark appropriately

Specimens

Viral isolation	Serum <input checked="" type="checkbox"/>	Blood in filter paper <input type="checkbox"/>	Whole blood <input type="checkbox"/>	Plasma <input checked="" type="checkbox"/>	Tissue <input checked="" type="checkbox"/>
Mac ELISA	Serum <input checked="" type="checkbox"/>	Blood in filter paper <input type="checkbox"/>	Whole blood <input type="checkbox"/>	Plasma <input checked="" type="checkbox"/>	
RT-PCR	Serum <input checked="" type="checkbox"/>	Blood in filter paper <input type="checkbox"/>	Whole blood <input checked="" type="checkbox"/>	Tissue <input checked="" type="checkbox"/>	
PRNT	Serum <input type="checkbox"/>	Blood in filter paper <input type="checkbox"/>	Whole blood <input type="checkbox"/>		
ELISA IgG	Serum <input checked="" type="checkbox"/>	Blood in filter paper <input type="checkbox"/>	Whole blood <input type="checkbox"/>		
Others (specify):	The use of blood in filter paper is currently under investigation for RT-PCR and serological tests.				

4. What isolation and typing techniques use to characterize and identify dengue virus?

Isolation system	Method or Protocol used
Cells C6/36 yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	
Cells AP-61 yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	
Viral Isolation in mosquitoes yes <input type="checkbox"/> no <input checked="" type="checkbox"/>	
Viral Typing by IF yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	
Isolation in Suckling Mice yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	
Other cells (please specify) yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	Vero E6

Viral Typing by ELISA	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>	
Others		
5. What specialized techniques to identify and characterize the dengue virus are carried out?		
Test		Protocol used
RT-PCR	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	Lanciotti et al, JCM. 1992.
RFLP	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>	
Nucleotide Sequencing	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	For epidemiological study and research project.
Other techniques *	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>	
* (genetic analysis)		
6. Do you currently use rapid diagnostic tests?		yes <input checked="" type="checkbox"/> no <input type="checkbox"/>
6.1 If so, what type of test?	Platelia Dengue NS1 Antigen test.	
6.2 Commercial manufacturer	Bio-Rad Laboratories	
6.3 At what level within the health system are they used?	This test is routinely used as a first intention test for the detection of acute infection by dengue virus.	
Networks		
7. Does your centre/laboratory have a network that receives specimens from hospitals, physicians, or laboratories?		yes <input checked="" type="checkbox"/> no <input type="checkbox"/>
7.1 If yes: How many laboratories are integrated into that network?	10	
7.2 What diagnostic capability do those laboratories have?		
Serological:	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	Techniques used: Commercial tests
Virological:	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>	Techniques used:
Molecular:	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	Techniques used: Lanciotti et al, JCM. 1992.
Observations:	Some of these laboratories are also using the Platelia Dengue NS1 Antigen test.	
7.3 Mention briefly the structure of the Network	When a laboratory is detecting a NS1 positive test on acute serum sample, this sample is sending to the National Reference Centre for identification of the type of dengue virus in charge of this infection by RT-PCR.	
7.4 Does the Network use an internal proficiency system?	yes <input checked="" type="checkbox"/> no <input type="checkbox"/>	
If yes, please give a brief description:	Since 2003, the National Reference Centre has been sending once a year a proficiency test for Dengue IgM (10 labs) and for Dengue RT-PCR (1 lab).	

7.5 Would you like your integrated laboratory network to participate into a program of International Quality Assurance for Diagnosis of Dengue organized by the WHO/PAHO Collaborating Centres in 2007?		yes <input checked="" type="checkbox"/> no <input type="checkbox"/>
If yes, state which part and provide the e-mail, address, and contact information for yourself and any other responsible parties.		
Centre National de Référence des Arbovirus Institut Pasteur de la Guyane 23, avenue Pasteur – BP 6010 97306 Cayenne Cedex FRENCH GUIANA jmorvan@pasteur-cayenne.fr pdussart@pasteur-cayenne.fr cnrarbo@pasteur-cayenne.fr Phone: +594.(0)594.29.26.01 Phone: +594.(0)594.29.26.09 Fax: +594.(0).594.30.94.16		
Quality Control		
8. Does the laboratory use an internal quality control program?		yes <input checked="" type="checkbox"/> no <input type="checkbox"/>
8.1 If so, describe it briefly		Evaluation once a year of the sensitivity of RT-PCR protocol using titrated viral suspension.
9. Does the laboratory participate in an international quality assurance program?		yes <input type="checkbox"/> no <input checked="" type="checkbox"/>
9.1. If yes, describe it briefly.		
9.2. If not, would it wish to participate in a program of International Quality Assurance for Diagnosis of Dengue organized by the WHO Collaborating Centres/PAHO in 2007?		yes <input checked="" type="checkbox"/> no <input type="checkbox"/>
Diagnostic Confirmation and Need for Reagents		
10. Does the laboratory send sera isolates and/or dengue virus isolates to WHO/PAHO collaborating centres or reference laboratories and/or to other specialized laboratories for serological or serotype confirmation or for other special characterization?		yes <input type="checkbox"/> no <input checked="" type="checkbox"/>
10.1 If yes, please indicate the name(s) of the specialized collaborating centre(s).		
11. Does the laboratory use reagents produced in the Collaborating Centres and reference labs for the diagnosis of dengue?		yes <input type="checkbox"/> no <input checked="" type="checkbox"/>
11.1 If yes, indicate the centre or reference laboratory		
12. Please indicate use of the following reagents and list Collaborating Centre or reference laboratory from which it is supplied.		
Antigens	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>	
Antiserum	yes <input type="checkbox"/> no <input checked="" type="checkbox"/>	

Monoclonal Antibodies	yes <input type="checkbox"/>	no <input checked="" type="checkbox"/>	
Cell cultures	yes <input type="checkbox"/>	no <input checked="" type="checkbox"/>	
Diagnostic Capability for Other Flaviviruses			
13. Yellow fever:	yes <input checked="" type="checkbox"/>	no <input type="checkbox"/>	Techniques: Cell culture, molecular biology, MAC-ELISA and indirect ELISA (for IgG detection)
14. West Nile:	yes <input checked="" type="checkbox"/>	no <input type="checkbox"/>	Techniques: Cell culture, molecular biology, MAC-ELISA and indirect ELISA (for IgG detection)
15. Saint Louis Encephalitis:	yes <input checked="" type="checkbox"/>	no <input type="checkbox"/>	Techniques: Cell culture, molecular biology, MAC-ELISA and indirect ELISA (for IgG detection)
16. Equine Encephalitis:	yes <input checked="" type="checkbox"/>	no <input type="checkbox"/>	Techniques: Molecular biology, MAC-ELISA and indirect ELISA (for IgG detection) for Tonate virus (VEEV subtype IIIb)
Others:	Detection of Mayaro and Chikungunya viruses using: cell culture, molecular biology, antibody detection (MAC-ELISA, indirect ELISA).		