

## DIAGNOSIS AND SURVEILLANCE OF DENGUE VIRUS INFECTIONS: GOLD STANDARDS AND NEW DIRECTIONS

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### INTRODUCTION

The dengue viruses belong to the family Flaviviridae, named after the prototype yellow fever virus, which has played a leading role in the development of the science of medical virology. Other important viruses in this family are the Japanese encephalitis virus, St. Louis encephalitis virus and tick-borne encephalitis virus. All these viruses are arthropod-borne viruses and are important causes of human disease.

The most important breakthrough in laboratory diagnosis of arboviruses was the development of the haemagglutination test which detected the presence of virus antigen.<sup>1,2</sup> The detection of antibody to viral haemagglutinin was now possible using a haemagglutination inhibition test with haemagglutinins prepared from the sera of infected monkeys, and thus began the tradition of the haemagglutination inhibition (HI) test. The much cited work of Clarke and Casals<sup>3</sup> on HI test methods made it possible to make confirmatory laboratory diagnoses of many arbovirus infections by demonstrating seroconversions in paired sera, and this has since been the "gold standard" of arbovirus serology.

This paper will discuss currently used methods of diagnosis of dengue virus infection and will review critically the many recently published variations of methods used for the determination of anti-dengue IgM. We will then take a brief look at future trends and needs in the development of tools for the diagnosis and surveillance of dengue virus infections.

### VIRUS ISOLATION

The most successful systems of dengue virus isolation have been by inoculation of mosquitoes by the intra-thoracic route.<sup>4,5</sup> Others have tried intra-cerebral inoculation of adult mosquitoes<sup>6</sup> or larvae.<sup>7</sup> All these methods involve the detection of antigen in head squashes by the immunofluorescence antibody technique, 5 to 14 days after inoculation. The necessary requirements of

maintaining a mosquito colony and an immunofluorescence microscope, as well as developing microinjection skills, limit the usefulness of these techniques as diagnostic tools in many laboratories.

The use of mosquito cell culture systems<sup>8</sup> or macrophage cell lines<sup>9</sup> suffer from the problem of sensitivity (in the case of mosquito cell lines) and the need for cell culture facilities and immunofluorescence techniques, which preclude the use of these methods on a wide scale.

Virus isolation is extremely important and useful for generating epidemiological information, but must remain, for the present, methods used in central, reference and research laboratories, and will not be discussed further in this paper.

### SEROLOGY

While the HI test remains the gold standard for the diagnosis of dengue infection, there are many associated problems which render it expensive and difficult to perform optimally. Haemagglutination is highly pH-sensitive and with every change of reagent, pH optimisation needs to be carried out for each haemagglutinin used. At least four haemagglutinins (Den 1 to 4) are used in titrations of paired sera taken 10 to 14 days apart, making it necessary to perform 8 titrations for each patient. This means that one 96-well microtitre plate is used for confirmatory diagnosis for each case, and the result can only be available at convalescence.

The need for paired sera is inconvenient and, in most hospitals, less than half the patients with a clinical diagnosis of dengue fever or dengue haemorrhagic fever return to have a second serum specimen taken. Thus most results of single specimens tend to be reported as inconclusive.

It has become necessary then, to develop a serological test which only requires a single specimen for diagnosis. The search has concentrated on the development of assays for the determination of dengue specific IgM.

**DETERMINATION OF IgM**

There have been many approaches to the problem of how best to determine the presence of dengue specific IgM in patients' sera. Radioimmunoassay (RIA) was once the method of choice, but today, the trend is towards the use of enzyme immunoassay (EIA). Table 1 is a summary of some of the main methods recently published on the determination of dengue virus specific IgM by EIA. The main focus has been on what is called the IgM capture methods, particularly the MAC ELISA (or IgM Antibody Capture ELISA). Due to the large amounts of dengue antigen-binding IgG which may be present, particularly in cases of secondary dengue, it is better to "capture" human  $\mu$  chains onto a solid phase. In this way, all IgM bound to the solid phase will have the opportunity to bind to antigen which will be added in the next step. The presence of dengue specific IgM is determined in one of 4 ways:

- i. enzyme-labelled antigen.
- ii. antigen, followed by labelled antibody to dengue.
- iii. antigen, followed by antibody to dengue, followed by labelled secondary antibody.
- iv. antigen (haemagglutinin), followed by goose-red blood cells; haemadsorption is looked for.

In methods (ii) and (iii), the label used has been  $^{125}\text{I}$  as well as an enzyme such as horseradish peroxidase. Methods (i), (ii) and (iii) are EIA's and RIA's, while (iv) is a solid phase immunosorbent test (SPIT).

Table 2 summarises results of the IgM capture EIAs used in confirmatory diagnosis, culled from the work of several groups, as referenced in the table. It is clear that the IgM-capture EIA is unable to pick up IgM in every acute phase serum of dengue patients. At best, in the Bangkok series<sup>12</sup> which also had the largest number of samples, IgM was seen in 78% of the acute phase sera of known dengue patients.

It should also be pointed out that the data from Lam's group<sup>13</sup> showed that the acute phase sera of cases with primary dengue were less likely to have dengue specific IgM (11/23) than cases with secondary dengue (12/15). Our own observations (Cardosa, unpublished) confirm this finding. IgM determination is therefore not entirely a satisfactory solution to the problem of rapid diagnosis of dengue fever and dengue haemorrhagic fever.

**DOT ENZYME IMMUNOASSAY**

Our approach to the problem of rapid diagnosis of dengue infections has been to use a highly sensitive dot enzyme immunoassay (DEIA) to determine the presence of IgG to dengue virus antigen.<sup>16</sup> By titrating acute and convalescent sera from febrile patients with dengue fever or dengue haemorrhagic fever, as well as sera from febrile patients proven not to have dengue fever or dengue haemorrhagic fever, we have shown that most patients positive for dengue infection by HI criteria will be positive at 1:1000 dilution in the DEIA, while most patients negative for dengue infection by HI will be negative in the DEIA.<sup>17</sup>

**TABLE 1  
METHODS OF DETERMINING FLAVIVIRUS SPECIFIC IgM**

Reference	Method
Schmitz & Emmerich <sup>10</sup>	IgM capture; enzyme labelled antigen and antigen followed by goose erythrocytes.
Burke, et al. <sup>1</sup> & Innis, et al. <sup>2</sup>	IgM capture; enzyme labelled antinflavivirus antibody.
Lam, et al. <sup>3</sup>	IgM capture; enzyme labelled antibody to monoclonal antinflavivirus antibody.
Gunasekaran, et al. <sup>14</sup>	IgM capture; antigen followed by goose erythrocytes.

5  
4  
3  
2

TABLE 2  
 PRESENCE OF IgM IN ACUTE PHASE SERA OF PATIENTS WITH CONFIRMED  
 DENGUE INFECTION

Reference	No. positive/total sample (%)
Burke <sup>1,5</sup>	15/40 (38)
Lam, et al. <sup>3</sup>	23/38 (61)
Innis, et al. <sup>2</sup>	924/1183 (78)
Cardosa, et al., unpublished	44/92 (48)

TABLE 3  
 COMPARISON OF DEIA AND HI FOR  
 DENGUE DIAGNOSIS

HI	DEIA	
	POSITIVE	NEGATIVE
POSITIVE	45	3
NEGATIVE	4	33

Table 3 shows a comparison of the DEIA with the HI test. 85 paired sera from patients with a clinical diagnosis of dengue fever or dengue haemorrhagic fever were titrated against the 4 dengue serotypes in the HI test. Of these, 48 cases were shown to be positive by HI, and 37 cases were negative with no seroconversion shown by HI. When we looked at the acute phase sera using DEIA at 1:1000 dilution of serum, we found that 45 of the 48 confirmed positives were positive by DEIA. The remaining 3 seroconverted and were shown to be positive by DEIA at 1:1000 dilution by convalescence. Of the 38 which were negative by HI, 33 were also negative by DEIA, but 4 were positive in the acute serum. Two of these four apparently false positives seroconverted in the DEIA test at convalescence. The DEIA is a simple and effective rapid diagnostic tool for the control and surveillance of dengue virus infection.

#### GENOMIC PROBES

There is no doubt that the use of genomic probes will be the future of rapid diagnosis of viral diseases, and the search for such useful tools for dengue diagnosis is underway. An example is the work of Henchal *et al*<sup>1,8</sup> who used a radiolabelled cDNA probe in a slot blot nucleic acid hybridization technique to detect as little as 11 plaque forming units

of Dengue 2 virus. We will, however, end our discussion of diagnosis of dengue with the tools currently available, leaving the future of cDNA probes for dengue to that time in the future when they can become a practical reality.

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