

Rapid dengue diagnosis and interpretation

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INTRODUCTION

Outbreaks of dengue fever (DF) and dengue haemorrhagic fever (DHF) pose a serious threat to more than 85 tropical and sub-tropical countries throughout the world. The World Health Organization has identified dengue to be one of the most under-reported diseases, with actual cases in the millions. It is also recognised as one of the most important and rapidly rising mosquito-transmitted infections in the world.

The alarming increase in cases may be due to many factors, including rapid population growth, expanding urbanization, rising economies, inadequate municipal water supplies, and difficulties in handling refuse disposal. These lead to an abundance of new breeding sites for the mosquito vectors of the disease, while human migration patterns disperse vectors and viruses into new areas. National public health authorities are often unable to deal successfully with dengue outbreaks and epidemics. Although a vaccine developed in Thailand against dengue viruses is on the horizon, it will take a few years before it becomes available.

DENGUE DIAGNOSIS

In recent years, there have been some changes in the clinical presentation of dengue and these will be dealt with in detail in subsequent papers. Because of this, the clinical diagnosis can be confusing to the uninitiated and this will affect the management of patients. Conventional laboratory methods are too time consuming to be of much use and newer rapid methods are attempts to overcome these problems. In addition, the results obtained in a shorter period of time will undoubtedly help in the confirmation of outbreaks and control of the spread of the disease.

There are three approaches for the laboratory diagnosis of dengue infection. These are:

- (a) Virus isolation
- (b) Serology
- (c) Detection of viral RNA

(a) Virus isolation

The recovery of dengue virus from blood or tissue specimen is the most conclusive way to demonstrate dengue infection. However, this is not an easy task to perform because the virus grows poorly in animals and cell cultures. The use of mosquito cell cultures such as AP/61 and C6/36 cells has improved the sensitivity of isolation but the time required to get a positive result may still take up to two weeks. Inoculation of adult mosquitoes for virus isolation has been successfully achieved^{1,2} and is the preferred method in several laboratories. In our WHO Centre, we have developed the inoculation of *Toxorhynchites splendens* larvae for isolation of dengue viruses.^{3,4} The method is relatively simple and the results available within 5 days as compared to the longer time required when adult mosquitoes are used.

In the past, the identification of the virus isolates required the use of specific antisera in the neutralization test. This is not only technically difficult, but laborious and time consuming. However, using specific monoclonal antibodies to each of the dengue serotypes and immunofluorescence, the results can be obtained within hours. Viruses isolated in cell cultures can be similarly identified. Using a combination of mosquito larvae inoculation and mosquito cell cultures for isolation, we have been able to monitor the circulating virus serotypes over the last few years.

Table 1 shows the distribution of dengue viruses from 1985 to 1992. In all the years except 1985, all four dengue serotypes have been circulating in the country. The predominant strain in 1985 and 1986 was dengue 3 and this was replaced by dengue 1 in 1987 and 1988. Dengue 2 became the predominant strain from 1989 to 1991 and was responsible for the large and severe dengue outbreaks in those 3 years. Dengue 2 was replaced by dengue 3 in 1992 but the outbreak remained severe.

It has been reported in several countries that dengue 2 gives rise to severe dengue infections.

TABLE 1: Circulating virus serotypes (1985-1992)

1985	DEN 3	DEN 4	DEN 2	
1986	DEN 3	DEN 1	DEN 4	DEN 2
1987	DEN 1	DEN 3	DEN 2	DEN 4
1988	DEN 1	DEN 2	DEN 4	DEN 3
1989	DEN 2	DEN 1	DEN 3	DEN 4
1990	DEN 2	DEN 1	DEN 3	DEN 4
1991	DEN 2	DEN 3	DEN 1	DEN 4
1992	DEN 3	DEN 2	DEN 4	DEN 1

We have confirmed this finding between 1989-1991 and in addition, we discovered that dengue 3 is equally virulent. Using rapid virus isolation method, it is possible to determine the predominant virus serotype before the dengue season takes hold, and thus predict disease severity.

(b) Serology

Most laboratories depend on serological diagnosis to confirm dengue infections. There are a number of serological methods in use but the gold standard is the haemagglutination inhibition (HI) test. A more rapid test is the detection of dengue IgM by enzyme-linked immunosorbent assay (ELISA). A commercial kit (Dengue Blot) is also available and each of these tests will be discussed.

Haemagglutination inhibition

This test is based on the ability of dengue antibody to inhibit dengue viruses from agglutinating certain species of red blood cells, e.g. goose erythrocytes. It is the most widely used serological test for dengue antibody. The recommended

technique is that of Clarke and Casals,⁵ which has been adapted to microtitration equipment.

Ideally, paired sera (S1, S2) should be tested against at least two dengue antigens which should include a broadly-reactive antigen. Prior to testing, the sera should be extracted with kaolin or with acetone to remove non-specific inhibitors and then absorbed with goose red blood cells to remove non-specific agglutinins.

Difficulty is often encountered with the collection of paired sera. This makes the interpretation of single serum difficult. The test itself is labour-intensive and takes 3 days to perform. Interpretation is often made difficult when clinicians fail to provide adequate case history, including the date of onset.

Interpretation of dengue antibody responses in the HI test is shown in Table 2 published by WHO in 1986.⁶ It is noted that the HI test will not only confirm a positive dengue infection but also provide a possible answer to whether the patient is having a primary or secondary infection based on HI antibody levels. The titre used by WHO to determine a secondary infection is 1:2560 but based on local findings, we use a titre of 1:1280.

IgM dengue ELISA

Anti-dengue IgM is produced transiently during both primary and secondary dengue infections and its detection in any single serum specimen indicates an active or recent infection.

There are several methods currently in use for dengue IgM detection.^{7,8} We have developed an in-house IgM dengue ELISA which compares favourably with the HI test.⁹ Over 6,000 samples have been tested by both methods to date and the

TABLE 2: Interpretation of dengue HI antibody responses

Antibody responses	S1-S2 interval	Convalescent titre (any dengue antigen)	Interpretation
2 4x rise	≥ 7 days	≤ 1:1280	Definite infection, primary
≥ 4x rise	Any specimen	≥ 1:2560	Definite infection, secondary
≥ 4x rise	< 7 days	≤ 1:1280	Definite infection, possible primary or secondary
No change	Any specimen	≥ 1:2560	Presumed infection, secondary
No change	≥ 7 days	≤ 1:1280	Not dengue
No change	< 7 days	≤ 1:1280	Uninterpretable
--	One specimen only	≤ 1:1280	Uninterpretable

Source: Dengue Haemorrhagic Fever: Diagnosis, treatment and control, WHO, Geneva (1986)

IgM ELISA is at least as specific and sensitive as the HI gold standard. The advantage of the IgM ELISA is that it can be performed within a few hours and in many samples, the first or acute specimen is already positive, especially in a secondary infection. However, in a primary infection, IgM may not appear until the seventh day of disease.

IgM antibody is known to persist for 2-3 months and a positive result should be interpreted with caution and against clinical diagnosis. If a single specimen is negative, a second specimen taken soon after the first or on the day the patient is discharged may become positive so that there is less need to recall the patient.

Since the HI test is tedious to perform, it is hoped that the IgM ELISA can replace it as a routine serological test. It is also possible to decentralize this test throughout the country.

Dengue Blot

Dengue Blot is a commercial kit which is modified from the more popular ELISA and in which viral antigens are bound onto nitrocellulose membrane.^{10,11} It has been independently evaluated in Malaysia, Singapore, Thailand, Indonesia and Japan.

In 1989, we were provided with two free Dengue Blot kits by the manufacturer in Singapore for evaluation. Based on our limited study, we concluded that the test has a sensitivity of almost 100% in secondary dengue infection but only 28.6% in primary dengue. We were surprised that 2 out of 7 serologically confirmed rubella samples which were HI and IgM dengue negative were Dengue Blot positive and suggested that more samples should be tested.

Other laboratories have reported similar findings in terms of sensitivity and the conclusion is that the kit is useful as a screening test, particularly in dengue endemic countries where a high proportion of infection is expected to be secondary dengue. Negative samples may need to be tested by other serological methods.

(c) Detection of viral nucleic acid

The polymerase chain reaction (PCR) offers the potential for highly sensitive and specific detection of dengue viral RNA. PCR allows the *in-vitro* enzymatic amplification of minute quantities of genetic material and has found increasing application for the detection of a number of pathogens, including dengue.¹²⁻¹⁵

We have started to investigate the potential of this technique to detect and identify dengue

viruses in patients' sera from which virus isolates have already been demonstrated. Thirty-three virus positive specimens have been tested and all correctly identified by PCR. Five other specimens from suspected dengue patients with negative isolation were similarly tested and two were positive by PCR, one strain being dengue 2 and the other dengue 3. PCR helped to confirm a number of dengue deaths when other methods were inconclusive. The test takes only a few hours to perform, and confirmation by using cDNA probes will take slightly longer.

CONCLUSION

There are various approaches which have been found to be useful in the laboratory diagnosis of dengue infections. They vary in terms of rapidity, sensitivity and specificity. For practical purposes, serological tests such as IgM ELISA would be the method of choice. Commercial kits are useful for rapid screening especially in endemic areas where the majority of cases are secondary infection. However, cost may prevent its wider use. Virus isolation is useful where facilities are available but is not really practical for routine diagnosis. However, the polymerase chain reaction should be seriously considered as a complementary test to virus isolation. It is useful in determining the virus aetiology in severe dengue cases as well as in dengue deaths.

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