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Dengue diagnosis made easy

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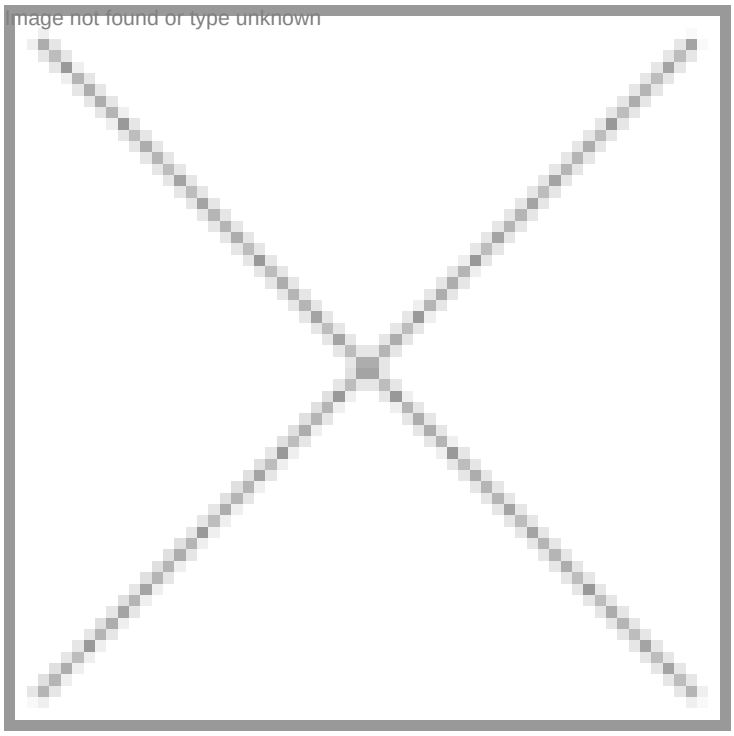
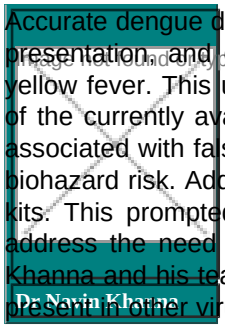


Currently, dengue fever is the most important re-emerging mosquito-borne viral disease, with the major proportion of the target population residing in the developing countries of the world. It is one of the leading public health concerns in over a hundred tropical and sub-tropical countries in South East Asia, the Caribbean, Central and South America. The World Health Organization (WHO) receives reports of about 5,00,000 dengue fever cases each year, but estimates that as many as 50 million people are infected annually.

Dengue is a mosquito-borne (Aedes aegypti), positive-stranded RNA virus of the genus Flavivirus. About 40 percent of the global population is estimated to be at risk of dengue infections. There are four antigenically distinct serotypes of dengue virus (DEN-1, 2, 3 and 4). The infection is accompanied with raging temperature and the agonizing limb pains that have earned the disease its sobriquet 'break-bone fever'. In addition, some patients lose hair and develop a measles-like rash, bleeding gums and depression that can last for weeks. In a small percentage of cases, mostly in infants, the infection causes dengue haemorrhagic fever (DHF) which is fatal.

Dengue diagnosis

Dr Navin Khanna, senior scientist and group leader, Recombinant Gene Products Lab, ICGEB, along with his team has developed a novel recombinant dengue multi epitope protein that exhibit potential for early detection of dengue infection.



Accurate dengue detection proves to be elusive as very often the diagnosis of dengue in endemic regions is based on clinical presentation, and can be confused with other viral diseases with similar clinical features like Japanese Encephalitis and yellow fever. This underlines the importance of laboratory-based diagnostic tests in providing timely medical attention. Most of the currently available commercial dengue diagnostic kits rely on the use of whole virus antigens and are consequently associated with false positives (due to serologic cross-reactivity with other flavi viruses), high cost of antigen production, and biohazard risk. Additionally, sera from patients with typhoid, malaria and leptospirosis also tend to score positive using these kits. This prompted the need to develop an alternate antigen to replace the whole virus antigen in diagnostic tests. To address the need for developing cost-effective, simple and rapid diagnostics that combines sensitivity and specificity, Dr Khanna and his team explored the option of synthetic protein antigens containing dengue virus specific epitopes that are not present in other viruses. The scientists chose 15 'unique' epitopes containing about 300 amino acids on the basis of pepscan analysis, phage display, and computer predictions, which were "stitched" together.

In this way, the group designed and expressed novel recombinant protein antigen by assembling key immunodominant, linear, conserved, dengue virus epitopes. The recombinant protein was expressed to high levels in the bacterium Escherichia coli, purified in a single step, yielding more than 25mg pure protein per litre culture. In order to detect its efficacy, the researchers then developed an in-house Enzyme-Linked ImmunoSorbent Assay (ELISA) to detect anti-dengue antibodies in a panel of 170 patient sera using the purified recombinant proteins as the capture antigen. The ELISA results were found to be in excellent agreement with those obtained using a commercially available diagnostic test. Efforts are being made to evaluate its performance with more sera samples.

Dr Khanna elaborated that since the epitopes were chosen very carefully, the chance of false positives was very less. "The high epitope density, careful choice of epitopes, and the use of E. coli system for expression, coupled to simple purification, jointly have the potential to lead to the development of an inexpensive diagnostic test with a high degree of sensitivity and specificity". Dr Khanna and his colleagues have got a patent on the diagnostic intermediate.

Dengue has been a neglected disease and is popularly known as Malaria's poor cousin, but the consciousness to combat is steadily increasing. The biggest bottleneck in developing a good kit is the lack of a well-characterized dengue sera panel. In fact Dr Khanna had to test the recombinant protein against the sera panel in Sri Lanka, as such a panel is practically non-existent in India. Now the Department of Biotechnology (DBT) has identified the need of a 'gold standard' sera panel against dengue in the country. Institutes like NICS (National Institute of Clinical Studies), NIV (National Institute of Virology), AIIMS (All India Institute of Medical Sciences), ICGEB (International Centre for Plant Engineering and Biotechnology), MAMC (Maulana Azad Medical College) are expected to spearhead this effort.

Dengue vaccine

The existence of multiple but distinct dengue virus serotypes is a major factor that has hindered vaccine development efforts. Available evidence indicates that immunity against an infecting serotype is life-long, whereas cross-protection against other serotypes is transient. Protection against only one or two dengue viruses could actually increase the risk of potentially fatal dengue haemorrhagic fever and dengue shock syndrome. Thus, sequential infection in endemic areas, where multiple serotypes co-circulate, has the potential to trigger life-threatening disease. Therefore, a safe and effective dengue vaccine should ideally be "tetravalent" or capable of providing solid and long-lasting immunity to all four serotypes. Currently, in the world six different virus-based vaccines are in various stages of development. Dr Khanna and his group are working on a DBT-funded project to develop a vaccine against dengue. They are investigating the possibility of developing sub unit vaccine candidates using yeast and adeno-viral vector systems. n

Rolly Dureha