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Mini Review

Dengue Diagnostics: Current Scenario

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There is an urgent requirement for specific, sensitive and inexpensive dengue diagnostic tools that can be used for clinical management, surveillance and outbreak investigations would permit early intervention to treat patients and prevent or control epidemics. Additionally, new techniques for the early detection of severe disease such as the use of biomarkers have the potential to decrease morbidity and mortality. Recent developments in rapid detection technologies offer the promise of improved diagnostics for case management and the early detection of dengue outbreaks. This short review summarizes the various diagnostics tests currently pursued.

Keywords: Dengue hemorrhagic fever, diagnosis, epidemics, PCR based approach, monoclonal antibodies, viral fever, therapy, vaccine

Dengue virus is currently one of the most important mosquito borne virus-affecting people in more than 100 countries globally (WHO, 1997; Castleberry and Mahon, 2003). The disease is endemic mostly in tropical and sub-tropical areas and an evolving concern to other countries in Europe and USA (Rodriguez-Tan and Weir, 1998; Malison and Waterman, 1983). Dengue fever is considered to be one of the significant emerging diseases in modern times with huge fatality both in terms of loss of life as well as economic impact particularly in developing countries. It is estimated that more than 3 billion people are at risk. Annually about 100 million cases of dengue fever and dengue hemorrhagic fever (DHF) are reported (Monath, 1994; Laue *et al*. 1999; Guzman and Kouri, 1996). The potential threat of dengue disease increasing manifold stems from increased travel to and from endemic areas and new dengue virus strains being more and more exposed to susceptible populations particularly children. Due to the rapid spread of both virus and hosts, dengue has become an epidemic and an urgent public health concern, globally (Guzman and Kouri, 1996; Lupi and Tyring, 2003). Dengue viruses are single stranded RNA viruses that belong to the family *Flaviviridae*. There are four serotypes, i.e. DEN-1, DEN-2, DEN-3 and DEN-4. Primarily the *Aedes aegypti* mosquitoes transmit these four serotypes to humans. Dengue virus is a positive-stranded encapsulated RNA virus wherein the genomic RNA is about 11 kb in size and comprises of three structural proteins (nucleocapsid protein (C), a membrane

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associated protein (M), and envelope protein (E)), and seven nonstructural (NS) protein. Infection with any of the four serotypes induces a life-long protective immunity to that particular serotype but confers only partial immunity against later infections by the other three serotypes (Rice *et al.* 1985; Leyssen *et al.* 2000). Secondary infection with various dengue virus serotypes is a major risk factor for the more severe and often fatal DHF and the Dengue Shock Syndrome (DSS) due to the phenomenon of antibody-dependent enhancement (ADE) (Halstead *et al.* 1973; Halstead, 1988).

There is no available anti-dengue vaccine or therapy for the treatment of dengue infection infection (Sabchareon *et al.* 2004). Development of a dengue vaccine is challenging primarily because it must be tetravalent so that it confers protection against all four serotypes and overcome ADE. Also there is no proper animal model for dengue vaccine trials. Some candidate vaccines are in various stages of clinical trials at the moment but an efficient, safe, low-cost vaccine is still some time away. Dengue infection can range has from asymptomatic infection to a more severe form of disease. There is also a possibility of additional complications in dengue cases such as hepatitis etc. (Mackenzie *et al*. 2004; Eltzov *et al.* 2010). The diagnostic tests must be able to address the infection at every level also differentiating between the serotypes simultaneously. Vector control efforts have also been extensively adopted in dengue endemic countries but results are far from satisfactory. There is an enormous potential and urgent requirement for rapid, inexpensive and sensitive diagnostic for detection of dengue so that timely palliative intervention can be pursued (Ananda Rao *et al*. 2005). There have been a variety of different diagnostic techniques currently being used. Some of the techniques commonly employed are virus isolation, Hemagglutination Inhibiton (HI), Plaque Reduction Neutralization Test (PRNT), polymerase Chain reaction (PCR) and Immunoassay for antibody detection (Vaughn *et al*. 1998, 1999; Palmer *et al*. 1999). Other novel technologies are aggressively pursued on the lines of better sensitivity, specificity, rapid and which would be cost effective. This mini commentary review existing and other diagnostic tools currently used for detection of dengue infection.

Current diagnostics for dengue virus infection

Simplified detection tests are very important for timely and accurate diagnosis of dengue for imparting proper care and treatment to the patients. As dengue is endemic in mostly developing countries which have to bear an enormous economic burden due to the disease, it is very important to develop a diagnostic test which would be low cost, sensitive, specific with least cross-reactivity, can be carried out with minimal training and above all the test must be rapid and can be performed at any setting (Blacksell, 2012). The most commonly used methods for detection of dengue virus infection are antibody detection assays, viral isolation and Polymerase Chain Reaction (PCR) based diagnostics. Virus isolation has been so far considered as a standard protocol in identifying dengue infection. Virus isolation is carried out from clinical samples using mosquito cell lines such as AP-61, C6/36, etc (Guzman and Kouri, 1996; Kuberski and Rosen, 1977a). Associating immunofluorescence techniques with dengue specific monoclonal antibodies does the virus identification. Inoculating mosquitoes is one of the better methods for virus isolation inoculation owing to its higher sensitivity (Kuberski and Rosen, 1977a, 1977b). Reports confirm that virus isolation from whole blood is more sensitive compared to serum. But the necessity of proper infrastructure is a major deterrent for the routine use of this method. Another method of detection of dengue

infection is the detection of dengue viral antigens. ELISA based assays that detect the dengue non-structural protein 1 (NS1) are the most common methods employed as high concentrations of NS1 have been in reported in patients with primary as well as secondary dengue infections up to 10 days post disease onset (Das *et al.* 2009). NS1 localizes on the virus infected cells and subsequently secreted into the blood. An assay comprising of polyclonal and monoclonal antibodies as capture and detection antibodies along with purified NS1 from dengue infected cells as a standard was developed. Analysis of clinical samples with this assay signified the methods efficacy and more assays were developed having this basic principle many of which are being used commercially. Detecting viral antigens by indirect immunofluorescence assays have also been studied wherein monoclonal antibodies and fluorescein conjugated anti mouse or anti human antibodies are used. A visual end point constitutes a positive or a negative result.

Multiple methods have been pursued for the serological detection of dengue specific antibodies that include the HI test, PRNT, etc. The IgM /IgG ELISA, based on a sandwich format and the HI test are commonly used serological assays for diagnosis of dengue virus infections (Palmer *et al*. 1999). In the past, the HI test was used differentiate between primary and secondary dengue virus infections but lately antibody capture assays have been more popular (Palmer *et al*. 1999). The HI test needs pretreatment to get rid of haemagglutination inhibitors and also the test is prone to flavivirus cross reactivity as well as serotype cross reactivity (Palmer *et al*. 1999). Then there was the Complement fixation (CF) test which when compared to other tests has higher sensitivity in detecting primary dengue infections but the CF antibodies are found only after a week for a short period of time (WHO, 1999). Antibody detection ELISAs has become significant assays for the detection of dengue virus infection, the key advantages being its sensitivity and specificity. A number of kits are available in the market based on this principle (Innis *et al*. 1989; Das *et al*. 2009; Bundo and Igarashi, 1985). The Nucleic acid sequence based amplification or NASBA is an RNA amplification method based on chemi-luminescence for detection of mRNA. This method has been successfully used to detect other pathogens such as malaria (Berndt *et al.* 2000; Schoone *et al*. 2000). This technology was then applied for the detection of dengue of all four serotypes and the results were comparable to a standard immune-fluorescent-based virus isolation assay (Teles *et al*. 2005).

Dengue RNA can also be detected in clinical samples from humans or in mosquitoes by reverse-transcription–polymerase chain reaction (RT-PCR) amplification technique. Dot blot immunoassay is yet another technique to diagnose dengue infection. The requirement for expensive infrastructure is a major drawback in the routine use of the methodology. Recombinant proteins domains of dengue virus serotypes were analyzed in strips to detect serotype specific antibodies in clinical samples and validated by PCR based diagnosis (Maniatis *et al*. 1982). Dengue virus antigens can also be visualized in samples with labeled monoclonal antibodies, end point visualization done with fluorescent dyes, enzymes, etc. Dengue Immunoglobulin G assays can be used for the diagnosis of earlier dengue infections as well as current infections if timely serum collection could be done. Assays are generally done using dilutions of each serum and used to determine a primary or a secondary infection. IgG based assays are very useful but it also has a major limitation that it is not serotype specific although the assay possess higher sensitivity than the HI assay. IgM based dengue diagnosis is also an useful tool commonly used in clinical settings. IgM

appears within 5 days of onset of illness and has both high specificity and sensitivity. The antigen purity is a major parameter in IgM based assays. There are a lot of commercial kits that use antigens from all the four serotypes in the assay thus determining any dengue infection and not being serotype specific. Since IgM circulates till about 3 months post onset of illness, this assay is not a proper validation of a current infection. Nonetheless, rapid assays based on this principle have been developed for point of care applications.

The continued and concerted effort in developing low cost and sensitive diagnostic applications for dengue is praiseworthy but more research is needed to overcome the many limitations of the existing technologies. Virus isolation is a time intensive process, requires skilled personnel and expensive infrastructure and cannot distinguish between a primary and secondary dengue virus infection. RNA amplification also requires expensive set up and technically trained personnel. The dengue antigen detection assays are simple, rapid and inexpensive but lacks the sensitivity of a viral isolation or nucleic acid amplification technique. Another important parameter is the proper validation and effectiveness of the various commercial assays particularly in the developing countries (Peeling *et al*. 2010). The need of the hour is to improve upon the existing technologies by minimizing the drawbacks and the simultaneous persuasion of new technologies such as the use of biosensors. A biosensor based on liposomal amplification was developed to diagnose serotype specific synthetic dengue sequence. Promising outcomes have been reported but a lot of research has to be done to take the technology to the next level. Biosensor based detection has the potential to be more sensitive than conventional assays but the technology needs to overcome constraints such as interference issues between electrochemical substances and the unpredictability of the biomolecule immobilization on the sensor surface which may impact both sensitivity and specificity.

Future direction and conclusion

Dengue diagnostics has made considerable progress with novel technologies being evolved and significant improvements made on the existing methods but there is still an enormous potential towards an ideal detection system that would be simple, inexpensive, rapid, sensitive and specific. The development of immunoassays based on NS1 antigen has been very significant and this has led to a number of commercial kits that are largely used around the world. The use of bi-specific antibodies in NS1 based immunoassays can significantly enhance the sensitivity of the existing assay due to their monovalency for an antigen as well as simultaneous binding to an enzyme. Bi-specific antibodies are engineered bi-functional molecules having dual binding specificities in a single entity. Bi-specific antibodies have been successfully exploited in the development of immunodiagnostic assays (Kreutz and Suresh, 1997; Kammila *et al*. 2008; Sarkar *et al.* 2012; Malabadi *et al*. 2012; Khan *et al*. 2011, 2012). Our laboratory has developed a sensitive low cost immunoassay employing bi-specific antibodies for the diagnosis of dengue. The assay has many positives such as specificity, inexpensive, rapid and can be performed in any point of care setting by personnel with minimal technical expertise without the use of complex instruments (Malabadi, 2008; Malabadi *et al*. 2010, 2011, 2012). Such novel diagnostic in combination with other existing technologies needs to be evolved to provide timely therapeutic intervention to tackle the fatal disease.

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References

Ananda Rao R, Swaminathan S, Fernando S, Jana AM, Khanna N (2005) A custom-designed recombinant multiepitope protein as a dengue diagnostic reagent. *Protein Expression and Purification.* **41**(1):136–147.

Blacksell SD (2012) Commercial Dengue Rapid Diagnostic Tests for Point-of-Care Application: Recent Evaluations and Future Needs? *Journal of Biomedical Biotechnology*. 2012: Article ID 151967, 12 pages, doi:10.1155/2012/151967.

Berndt C, Muller U, Bergmann F, Schmitt U, Kaiser R, Muller C (2000) Comparison between a nucleic acid sequence-based amplification and branched DNA test for quantifying HIV RNA load in blood plasma. *Journal of Virological Methods*. **89**(1-2): 177-81.

Bundo K, Igarashi A (1985) Antibody-capture ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. *Journal of Virological Methods*. **11**:15–22.

Burke DSA Nisalak MA (1982) Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal **fl**uid. *Journal of Clinical Microbiology*. **15**:1034–1042.

Castleberry JS, Mahon CR (2003) Dengue fever in the Western Hemisphere. *Clinical Lab Science*. **16**: 34–38.

Das D, Mongkolaungkoon S, Suresh MR (2009) Super induction of dengue virus NS1 protein in *E. coli*. *Protein Expression and Purification*. **66**(1): 66-72.

Eltzov E, Atias D, Gheber L, Marks RS (2010) Dengue Virus Diagnostics in Detection of bacteria, viruses, parasites and fungi (ed. Mariapia Viola-Magni), Springer Publishing, Berlin, NATO Science for Peace and Security Series A: Chemistry and Biology, 2010,DOI 10.1007/978-90-481-8544-3_12.

Guzman MG, Kouri G (1996) Advances in dengue diagnosis. *Clinical Diagnostic Laboratory Immunology*. **3**:621–627.

Halstead SB, Shotwell H, Casals J (1973) Studies on the pathogenesis of dengue infection in monkeys. II. Clinical laboratory responses to heterologous infection. *Journal of Infectious Disease.* **128**:15–22.

Halstead SB (1988) Pathogenesis of dengue: challenge to molecular biology. *Science*. **239**:476–481.

Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P, Hoke CH (1989) An enzyme linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *American. Journal of Tropical Medicine. Hygiene.* **40**: 418–427.

Kammila S, Das D, Bhatnagar PK, Sunwoo HH, Zayas-Zamora G, King M, Suresh MR (2008) A rapid point of care immunoswab assay for SARS-CoV detection. *Journal of Virological Methods*. **152**(1–2):77–84.

Khan SR, Ganguly A, Malabadi RB, Sunwoo HH, Parashar A, Teixeira da Silva JA, Suresh MR (2011) Targeting strategies and nanocarriers in vaccines and therapeutics. *Research in Biotechnology*. 2(6):08-20.

Khan SR, Ganguly A, Malabadi RB, Sunwoo HH, Suresh MR (2012) Gene delivery system: A developing arena of study for new era of medicine. *Recent Patents on DNA & Gene Sequences*. 6(1): 2-9 (8).

Kreutz FT, Suresh MR (1997) Novel bispecific immunoprobe for rapid and sensitive detection of prostate-specific antigen. *Clinical Chemistry*. **43**(4):649–656.

Kuberski TT, Rosen L (1977a) A simple technique for the detection of dengue antigen in mosquitoes by immunofluorescence. *American Journal of Tropical Medicine Hygiene.* **26**:533-7.

Kuberski TT, Rosen L (1977b) Identification of dengue viruses, using complement fixing antigen produced in mosquitoes. *American Journal of Tropical Medicine Hygiene* **26**:538-43.

Laue T, Emmerich P, Schmitz H (1999) Detection of dengue virus RNA in patients after primary or secondary dengue infection by using the TaqMan automated amplification system. *Journal of Clinical Microbiology*. **37**: 2543–2547.

Leyssen P, Clercq ED, Neyts J (2000) Perspectives for the treatment of infections with Flaviviridae. *Clinical Microbiology Review*. **13**:67–82.

Lupi O, Tyring SK (2003) Tropical dermatology: viral tropical diseases. *Journal of American Academy of Dermatology*. **49**: 979–1000.

Mackenzie JS, Gubler DJ, Petersen LR (2004) Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nature Medicine*. **10**(Suppl 12): S98–109.

Malabadi RB, Ganguly A, Teixeira da Silva JA, Parashar A, Suresh MR, Sunwoo HH (2011) Overview of plant-derived vaccine antigens: Dengue virus. *Journal of Pharmacy and Pharmaceutical Sciences.* **14**:400-413

Malabadi RB, Ganguly A, Sunwoo HH, Suresh MR (2012) Role of bi-specific monoclonal antibodies in immune-diagnostic assay. *Research in Pharmacy.* **2**(3): 08-14.

Malabadi RB, Parashar A, Ganguly A, Suresh MR (2010) Expression of Dengue virus envelope protein in a different plant system. Faculty Research and Development day, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada, 19th November 2010. Pp - 31.

Malabadi RB (2008) Production of edible vaccines for oral immunization in transgenic plants, current and future prospective. *Journal of Phytological Research*. 21(1): 1-10.

Malison MD, Waterman SH (1983) Dengue fever in the United States: A report of a cluster of imported cases and review of the clinical, epidemiologic, and public health aspects of the disease. JAMA. **249**: 496–500.

Maniatis T, Fritsch EF, Sambrook J(1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1982.

Monath TP (1994) Dengue: the risk to developed and developing countries. *Proceedings of National Academy of Sciences USA*. **91**: 2395–2400.

Palmer CJ, King SD, Cuadrado RR, Perez E, Baum M, Ager AL (1999) Evaluation of the MRL diagnostics dengue fever virus IgM capture ELISA and the PanBio Rapid Immunochromatographic Test for diagnosis of dengue fever in Jamaica. *Journal of Clinical Microbiology*. **37**(5): 1600–1601.

Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S, Enria DA, Farrar J, Gubler DJ, Guzman MG, Halstead SB, Hunsperger E, Kliks S, H.S. Margolis HS, Nathanson CM, Nguyen VC, Rizzo N, Vazquez S, Yoksan S (2010) Evaluation of diagnostic tests: dengue. *Nature Review in Microbiology*. 12 (Suppl.), pp. S30–S38.

Rice CM, Lenches EM, Eddy SR, Shin SJ, Sheets RL, Strauss JH (1985) Nucleotide sequence of yellow fever virus: Implications for flavi virus expression and evolution. *Science*. **229**:726–733.

Rodriguez-Tan RS, Weir MR (1998) Dengue: a review. *Tex Medicine*. **94**: 53–59.

Sabchareon A, Lang J, Chanthavanich P, Yoksan S, Forrat R, Attanath P, Sirivichayakul C, Pengsaa K, Pojjaroen-Anant C, Chambonneau L, Saluzzo JF, Bhamarapravati N (2004) Safety and immunogenicity of a three dose regimen of two tetravalent live-attenuated dengue vaccines in five- to twelve-year-old Thai children. *Pediatr Infectious Disease Journal*. **23**(2): 99–109.

Sarkar S, Tang XL, Das D, Spencer JS, Lowary TL, Suresh MR (2012) A bispecific antibody based assay shows potential for detecting tuberculosis in resource constrained laboratory settings. *PLoS ONE*. **7**(2): e32340. doi:10.1371/journal. pone.0032340.

Schoone GJ, Oskam L, Kroon NC, Schallig HD, Omar SA (2000) Detection and quantification of Plasmodium falciparum in blood samples using quantitative nucleic acid sequence-based amplification. *Journal of Clinical Microbiology*. **38**:4072–4075.

Teles FRR, Prazeres DMF, Lima-Filho JL (2005) Trends in dengue diagnosis. *Review in Medical Virology*. **15**: 287–302.

Vaughn DW, Nisalak A, Kalayanarooj S, Solomon T, Dung NM, Cuzzubbo A, Devine PL (1998) Evaluation of a rapid immunochromatographic test for diagnosis of dengue virus infection. *Journal of Clinical Microbiology*. **36**(1):234–238.

Vaughn DW, Nisalak A, Solomon T, Kalayanarooj S, Nguyen MD, Kneen R, Cuzzubbo A, Devine PL(1999) Rapid serologic diagnosis of dengue virus infection using a commercial capture ELISA that distinguishes primary and secondary infections. *American Journal of Tropical Medical Hygiene*. **60**(4):693–698.

World Health Organization (1997) Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control, 2nd edn. World Health Organization: Geneva, 1997.

World Health Organization (1999) Regional Guidelines on Dengue/DHF Prevention and Control. Regional Publication 29/1999. World Health Organization: Geneva.