REVIEW

Progress in the diagnosis of dengue virus infections and importance of point of care test: A Review

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Abstract: It is an urgent need of highly sensitive, specific and economical diagnostic tools for early and fast diagnosis of highly challenging dengue virus infections. Many laboratory methods including virus detection, genome detection, antigen detection and serological detection of such short-lived viremia were explored but promising outcomes for economical immunochromatographic tests have been reported in this review. With the trend of fast, easy operation, rapid diagnostic tests (RDT) based on immunochromatographic assays are of great importance due to point of care test (POCT) in the dengue endemic regions where it is short of laboratory equipments and cold storage conditions. Such kind of point of care diagnosis is more efficient, fast and user friendly. Moreover, the development of highly advance RDT is dependent on the use of anti-dengue monoclonal antibodies highly specific for particular analyte/antigen.

Keywords: Dengue, diagnosis, rapid diagnostic tests, antibodies.

INTRODUCTION

Dengue is emerging as an enduring global threat in the tropical and subtropical developing countries of the world. This arthropod-born viral disease causes millions (50-100 million) of infections each year. World Health Organization (WHO) recently estimated that the prevalence of dengue has increased 30-folds compared with the situation 50 years before, thus affecting both human health and the economy (WHO, 2012). The major factors contributing to the spreading dengue viruses include the expanding urban population and environment, an increase in mosquito vectors density and the ease and frequency of rapid air travel allowing the daily movement of viremic individuals (Chien *et al.,* 2006; Gubler, 1999; Gubler and Clark, 1995). Dengue virus (*Flaviviridae* family) is transmitted to human by mosquitoes *Aedes aegypti* or *Aedes albopictus.* The incidence of all types of dengue viruses (serotypes 1-4) causes primary and secondary infections. Primary infection causes an acute febrile illness commonly identified as dengue fever, which is cleared in around seven days by a complex immune response while secondary infection causes dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). DHF and DSS are more lethal and differentiated by high fever, bleeding, thrombocytopenia as well as haemoconcentration. Common symptoms of dengue patients after 3-4 days onset of fever are: rash, petechiae, epistaxis and gingival as well as gastrointestinal bleeding. Low pulse rate or hypotension, plasma leakage, cold and moist skin and disturbed mental conditions can also be observed in severe infection (Pan American Health Organization, 1994; Nimmannitya, 1987; Martinez, 1992; Halstead, 1992).

Dengue virus is positive-sense single-stranded RNA virus, consists of a single open reading frame (approximately 11 kb) encoding a polyprotein precursor, which is proteolytically cleaved into capsid (C), premembrane (prM) and envelope (E) proteins (structural proteins) while seven nonstructural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) are also observed. Replication and assembly of virus depend on the accurate cleavage of the viral protein (Rice *et al.,* 1985; Cahour *et al.,* 1992). There are several evidences that these proteins especially NS1 play an important part in different stages of the virus life cycle and contribute a vital role in RNA replication and various cellular functions (Welsch *et al.,* 2009; Mackenzie *et al.,* 1996; Lindenbach and Rice, 1997; Muylaert *et al.,* 1997; Westaway *et al.,* 1997). Dengue viremia is very short and within few hours after infection, thousands copies of viral molecules are produced from a single viral molecule, which causes cell damage as well as becomes lethal. RNA polymerases and other cellular factors are involved in catalyzing the infection cycle to dengue virus (Hidari and Suzuki, 2011; Filomatori *et al.,* 2006).

This review is focused on the potential of commonly used different laboratories methods for diagnosis of dengue virus infections and importance of immuno-chromatographic assays with their future needs.

Diagnosis of dengue infection

With the increasing global incidence of dengue disease due to short viremia and the absence of effective vaccines

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for its prevention, rapid, economical and accurate diagnosis of infections during the first few days of clinical symptoms is crucial to provide proper treatment for the patient and early public health control of dengue outbreaks (Andries *et al.,* 2012; Tang and Ooi, 2012). Recently WHO stated that morbidity and mortality from dengue infections can be reduced to almost zero by the implementation of timely, appropriate clinical management, which involves early clinical or laboratory detection, proper staff training, intravenous rehydration and hospital reorganization (WHO, 2012). Diagnosis of dengue infection has always been a great challenge due to short life cycle of virus. Following laboratory methods are generally adopted for diagnosis of dengue infections.

Virus detection

The dengue virus was isolated for the first time from the blood samples of patient in Japan in 1943 by Kimura and Hotta (1943). Four isolation methods for dengue virus detection have been employed: mosquito inoculation (*Aedes aegypti* or *Aedes albopictus*) (Rosen and Gubler, 1974**;** Jarman *et al.,* 2011), mosquito cell cultures using C6/36 lines or AP61 (Chua *et al.,* 2011; Oliveira *et al.,* 2003; Tesh, 1979; Kuberski and Rosen, 1977), mammalian cell culture using vero cells or LLC-MK2 cells (Matsumura *et al.,* 1971; Yuill *et al.,* 1968) and intracerebral inoculation of suckling mice (Meiklejohn *et al.,* 1952; Lee *et al.,* 2005). Although virus isolation methods provide high sensitivity and specificity (table 1), but there are few limitations, which include long time (about 7 days), acute samples (0-5 days post onset), experienced staff, appropriate facilities and high cost. Consequently, this approach is not commonly used in routine diagnostic laboratories.

Genome detection

As cell culture based detection methods usually require seven days, reverse transcriptase polymerase chain reactions (RT-PCR) were developed for rapid genome detection and typing of dengue viruses (Lanciotti *et al.,* 1992). Usually, RT-PCR need one day for operation and has been confirmed as a valuable research tool in pathogenesis and molecular studies. RT-PCR is also commonly used for dengue virus (DENV) RNA detection in infected cells, serum, plasma, infected mosquito larvae, fresh and paraffin-embedded tissues and formalin-fixed tissue. Sensitivity and specificity of the conventional RT-PCR in different detections ranges from 48.4-100% and 100%, respectively (Lanciotti *et al.,* 1992; Raengsakulrach *et al.,* 2002; Chua *et al.,* 2011). Later, real time RT-PCR for diagnosis and quantification of dengue virus was developed and its sensitivity ranged from 58.9-100% and specificity was 100% (Shu *et al.,* 2003; Lai *et al.,* 2007; Gurukumar *et al.,* 2009; Pok *et al.,* 2010; Hue *et al.,* 2011; Waggoner *et al.,* 2013). The dengue virus isolation rate was also improved by nested PCR and mostly it was up to 79.4% (Jarman *et al.,* 2011). Nucleic acid sequence based amplification assay (NASBA) is another method to detect the dengue viral RNA from the whole blood or serum from infected patients in the acute phase (Wu *et al.,* 2001) and it showed high sensitivity (98.5%) and specificity (100%). Moreover, RNA extraction from whole blood was found more sensitive (90%) as compared to plasma/serum (62.0%) in the same pool of sample (Klungthong *et al.,* 2007). RNA detection methods can confirm the infections within 24-48 hours with high sensitivity and specificity. Identification of serotype and genotype can also be performed. But these methods require acute phase samples, expensive laboratory equipments and trained technicians. False positive results are also frequently observed due to sample contamination (Peeling *et al.,* 2010).

Antigen detection

Among all virus antigens, NS1 was found at the concentration of several micrograms per milliliters in the serum of infected patients from the start of fever up to the ten days (Young *et al.,* 2000; Alcon *et al.,* 2002; Alcon-Le Poder *et al.,* 2006). The quantity of NS1 circulating in the blood of DHF infected patients was found to be significantly higher than dengue fever patients (Libraty *et al.,* 2002). The maximum diagnosis of NS1 antigen can be obtained between days 3 and 5 in both kinds of primary and secondary infections. All these findings indicate that NS1 is an attractive marker for the diagnosis of infection (Alcon *et al.,* 2002).

Antigen detection is considered to be more important and highly sensitive method compared with others at present. For dengue viral antigens detection, immunofluorescence assay (IFA) and radioimmunoassay (RIA) can not be used for routine diagnosis tests because of low sensitivity (Monath *et al.,* 1986). Dengue antigen detection by immunohistochemical techniques (using alkaline phosphatase labels or horseradish peroxidase) (Pelegrino *et al.,* 1997) is also used but not effective. Young and co-workers (2000) firstly successfully standardized a capture NS1 ELISA for NS1 detection in serum during the acute phase of secondary infection. The availability of commercial kits in the markets for dengue NS1 antigen diagnosis in acute serum provides an alternative to the existing methods such as virus isolation, PCR and serology. Some commercial kits based on ELISA format are also available in markets. Panbio (Australia), Biorad (France), and Standard Diagnostics (SD) Bioline (South Korea) are the major companies for manufacturing the commercial rapid diagnostic tests (RDT) for NS1 antigen tests based on ELISA assays. Many studies were performed to evaluate these commercial kits (Kumarasamy *et al.,* 2007; Bessoff *et al.,* 2008; Chaterji *et al.,* 2011; Puttikhunt *et al.,* 2011; Blacksell *et al.,* 2012), which showed in table 1. The sensitivities observed in these studies ranged from 94.7-98.3% for

primary infections and 67.1-77.3% for secondary infections while specificities were 100%. However, the NS1 detection was less sensitive in secondary infections as compared to primary infections. The lower NS1-antigen detection in dengue secondary infection can be attributed to NS1 antigen complexing with anti-NS1 antibodies (Young *et al*., 2000; Libraty *et al*., 2002). These ELISA format rapid diagnostic tests employ serotype-specific anti-NS1 monoclonal antibodies to capture and detect soluble NS1 antigen in serum, plasma, or blood. Antigen detection assays based on ELISA can confirm the infection easily and are less expensive. But it is not as sensitive as virus isolation or RNA detection methods.

Serological detection

Serological testing is the widely adopted method in the hospitals for the diagnosis of dengue infections. Serology is a valuable support in the diagnosis of dengue infections (Lam, 1993; Lam, 1995). Traditionally, hemagglutination inhibition (HAI) assays have been used for the diagnosis of dengue (Vaughn *et al.,* 2000). Complement fixation (CF) and neutralization test (NT) are also used for diagnosis but not very common (WHO, 1986; Clarke and Casals, 1958).

For proper and timely treatment of dengue-infected patients, it is compulsory to diagnose the primary and secondary infections accurately. Mostly, hospital laboratories and commercial kits manufacturers defined acute primary dengue virus infection as an IgM positive and IgG negative and acute secondary dengue virus infection as IgM positive and IgG positive or IgM negative and IgG positive. But authenticity of such claims was only confirmed by several studies (Blacksell *et al.*, 2006; 2007; 2011).

Innis *et al.* (1989) demonstrated that stronger and specific IgM antibody isotype was the first immunoglobulin to emerge in the primary infections while anti-dengue IgG appears at the end of the first week of clinical symptoms in a low titer and then increased slowly. On the other hand, antibody titers increase rapidly during a secondary infection and generally antibody reacts with other flaviviruses. In the acute phase of infection, IgG is detectable with high levels following by the considerable rise during the next two weeks. False negative reactions of anti-dengue IgM can also be observed in secondary infections. Enzyme-linked immunosorbent assays (ELISA) especially MAC-ELISA corresponds to the most important advances for IgM detection for routine dengue diagnosis. Other formats like dot-ELISA, capture ELISA, AuBioDOT IgM capture and capture ultramicro-ELISA, GAC-ELISA for IgG are also developed (Innis *et al.,* 1989; Nawa *et al.,* 2001; Vázquez *et al.,* 1998). ELISA based kits for anti-dengue IgM and IgG detection are also commercially available prepared by different companies (Dengue virus IgM ELISA and IgG ELISA by Standard Diagnostics, South Korea; Panbio dengue virus IgM capture ELISA, Panbio dengue virus IgG capture ELISA by Alere, Australia etc.). Several studies used these commercial kits for the evaluation of these commercial kits (Blacksell *et al.,* 2012; Berde *et al.,* 2012; Hunsperger *et al.,* 2009; Chua *et al.,* 2011; Groen *et al.,* 2000). The ranges of sensitivities for IgM and IgG ELISA kits were observed from 20.22-99% and 7.8-88.9%, respectively (table 1) while specificities ranged from 52-100% for IgM and 63.5-100% for IgG. Due to the cross-reactivity of IgM with other flaviviruses such as yellow fever and Japanese encephalitis, dengue serotype determination is not reliable (Vázquez *et al.,* 2003). Presently, anti-dengue IgM and IgG detection based on ELISA is generally used to differentiate between the primary and secondary infection. Because IgM and IgG show the cross action with other flavivirus diseases, this method is not very successful and false positive results are obtained.

Improvement of the diagnostic tests

All above-mentioned protocols represent many advances in dengue diagnosis and are performed in the laboratories with different equipments and tools for many years. However, these methods are time consuming, costly, requires specific laboratory equipment and experienced technicians. The global prevalence of dengue infections is remarkably increasing especially in the developing countries where it is in short of laboratories facilities. In such dengue endemic condition, development of more sensitive, specific, cheap and easy tests are still necessary for early dengue diagnosis.

Importance of the immunochromatographic assays

Presently, more advanced biosensors are developed and used extensively in diverse fields like forensic, medical or environmental areas (Nakamura and Karube, 2003). Among all those biosensors or assays, improvements of immunochromatographic assays using monoclonal antibodies as ligands, are of significance. These assays can be used easily in dengue endemic regions especially in the developing countries with limited or no laboratory resources and no cold storage facilities. The immunochromatographic assay consists of a membrane strip acts as the immunosorbent. This strip is very useful for point of care tests (POCT) with fast, cheap and one step analysis. The essential features of this assay include user-friendly operation and short time for analysis of results (Vaughn *et al.,* 1998; Sang *et al.,* 1998). For immunochromatographic assay, tests can be performed without using any reagents like ELISA and person without any expertise can perform it. This assay is also known as one-step assay, which speed up the analytical procedure. Due to all above-mentioned properties, this one step assay can be now broadly used as a suitable system for the detection of dengue infections especially in developing countries.

Table 1: Description of different types of commonly used laboratory diagnostic tests for dengue infections

Type	Methods	Sensitivity (%)	Specificity (%)	References
Virus Detection	Virus isolation by mosquitoes	71.5-84.2	100	Jarman *et al.*, 2011; Kuberski and Rosen 1977
	Virus isolation by cell culture (C6/36)	40.5	100	Chua *et al.*, 2011
Genome Detection	RT-PCR (RNA detection)	48.4-100	100	Lanciotti *et al.*, 1992; Raengsakulrach *et al.*, 2002; Chua *et al.*, 2011
	Real time RT-PCR (RNA detection)	58.9-100	100	Waggoner *et al.*, 2013; Hue *et al.*, 2011; Pok *et al.*, 2010; Lai *et al.*, 2007; Chien *et al.*, 2006
Antigen Detection	ELISA Kits (NS1 detection)	94.7-98.3for primary infections and 67.1-77.3for secondary infections	100	Kumarasamy*et al.*, 2007; Bessoff *et al.*, 2008; Chaterji *et al.*, 2011; Puttikhunt *et al.*, 2011; Blacksell *et al.*, 2012
Serological Detection	ELISA for IgM detection	20.22–99.0	52-100	Hunsperger *et al.*, 2009; Groen*et al.*, 2000; Chua *et al.*, 2011; Blacksell *et al.*, 2012; Berde *et al.*, 2012
	ELISA for IgG detection	7.8-88.9	63.5-100	Groen *et al.*, 2000; Hunsperger *et al.*, 2009; Chua *et al.*, 2011; Blacksell *et al.*, 2012; Berde *et al.*, 2012
Immunochromatographic assays based detection with famous commercial kits	SD Bioline Dengue Duo kits for IgM detection	53.5-79.2	89.4-100	Wang and Sekaran, 2010; Blacksell *et al.*, 2011; Tontulawat *et al.*, 2011
	SD Bioline Dengue Duo kit for NS1 detection	44.4-70.6	73.4-100	Ferraz *et al.*, 2013; Andries *et al.*, 2012; Tontulawat *et al.*, 2011; Blacksell *et al.*, 2011; Osorio *et al.*, 2010; Wang and Sekaran, 2010; Tricou *et al.*, 2010
	SD Bioline Dengue Duo kit of NS1, IgM and IgG combinationdetection	75.5-92.9for NS1/IgM; 80.7-85.7for NS1/IgM/IgG	88.8-100 for NS1/IgM; 83.9-97.9 for NS1/ IgM/IgG	Andries *et al.*, 2012; Blacksell *et al.*, 2011; Osorio *et al.*, 2010; Wang and Sekaran, 2010; Tricou *et al.*, 2010
	Panbio Dengue Duo cassette for IgM or IgG detection	54.5-81.8 for IgM; 62.1-87.5 for IgG	75.0-97.6 for IgM; 66.6-94.4 for IgG	Blacksell *et al.*, 2006; Nga *et al.*, 2007; Moorthy *et al.*, 2009; Blacksell *et al.*, 2011; Pan-ngum *et al.*, 2013
	Panbio Early Rapid NS1	45.9 -88.06	92.5-100	Fry *et al.*, 2011; Blacksell *et al.*, 2011; Ferraz *et al.*, 2013; Pan-ngum *et al.*, 2013
	Panbio Early Rapid NS1 and Duo assay kit for detection of NS1, IgM, and IgG combination	89.0-89.9for NS1/IgM; 87.0-93.0for NS1/IgM/IgG	75.0 for NS1/IgM; 82.8 for NS1/IgM/IgG	Fry *et al.*, 2011; Blacksell *et al.*, 2011; Pan-ngum *et al.*, 2013

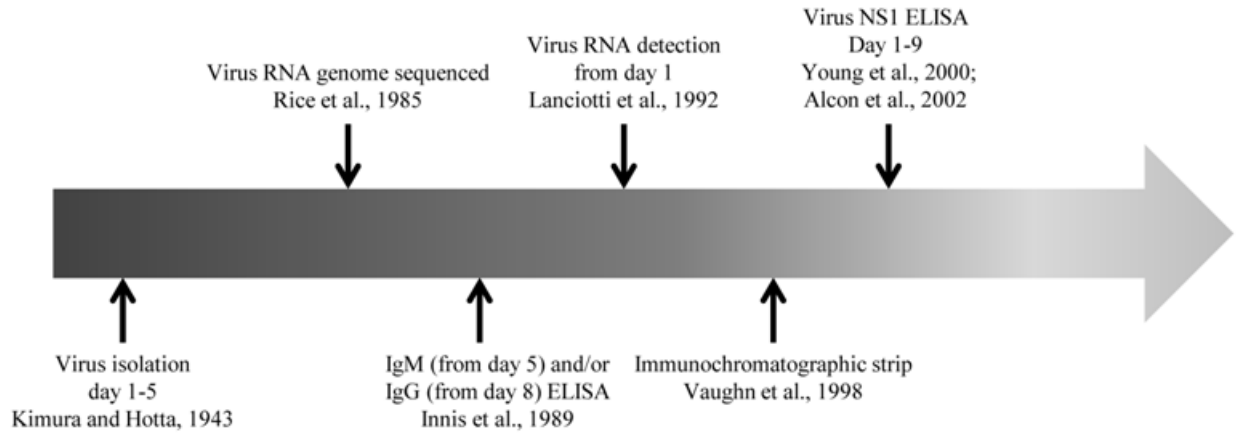

Fig. 1: History of diagnosis of infections with dengue virus. This time line represents history of detection methods with their inventors and specific time period of each method required for detection. Although, sequencing of Virus RNA genome is not detection method but it promoted method development for diagnosis of dengue virus infections.

The activity of immunochromatographic assay is dependent on the ligands. These ligands are highly specific monoclonal antibodies (MAbs) against dengue virus antigen. When blood sample of dengue infected patient containing antigen (analyte) is allowed to run, the analytical signals come out within 15-20min in the form of red colored lines after the particular interaction of ligand (antibody) and analyte (antigen). The capillary action mechanism is involved for this kind of interaction occurred on the membrane. Such interaction of antigen to antibody can be monitored fastly, effortlessly and with high sensitivity (Vaughn *et al.,* 1998; Sang *et al.,* 1998).

Evolution of dengue rapid diagnostic test based on immunochromatographic assay

Few rapid diagnostic kits based on immuno-chromatographic assays for dengue NS1 antigen, IgG, IgM and IgA antibodies detection are developed by some companies with higher specificities and sensitivities as compared to ELISA based RDTs. Immuno-chromatographic tests (ICT) for IgM, IgG or IgA antibodies or NS1 antigen detection have been existed in different forms for more than one decade (Standard Diagnostics BIOLINE Dengue Duo NS1antigen and IgG and IgM Combo Device for NS1 Ag IgM/IgG analytes, Alere Panbio Dengue Early Rapid Kit for NS1 Ag analyte, Alere Panbio Dengue Duo cassette for IgM/IgG analyte, Biorad STRIP for NS1 Ag analyte, MP Diagnostics ASSURE for IgA analyte).

Dengue RDT/ICT based on IgG detection has been forbidden due to the enduring persistence of dengue IgG antibodies in human blood, which gives false positive results. So, the first generation of kits for IgM, IgG or IgA antibodies detection developed by the above mentioned commercial companies had lower sensitivity and specificities, which is also mentioned by Blacksell *et al.* (2012). Therefore, we compared three kinds of kits developed by Standard Diagnostics BIOLINE and Panbio commercial companies with latest references (table 1).

In the recent years, the most important development for the diagnosis of dengue infections was the advent of the specific detection of NS1 antigen in the blood or serum samples of dengue-infected patients. As NS1 was found to be an interesting target due to its presence from the first day of infection, next generation of ICTs for NS1 antigen detection showed better efficiency compared with the first generation of kits (table 1). The ICTs developed by SD Bioline for NS1 detection showed sensitivity ranged from 44.4-70.6% and specificity ranged from 73.4 to 100% while sensitivity and specificity for ICTs developed by Panbio was ranged from 45.9-88.06% and 92.5-100%, respectively (Andries *et al.,* 2012; Blacksell *et al.,* 2011; Tricou *et al.,* 2010; Wang and Sekaran, 2010; Osorio *et al.,* 2010; Tontulawat *et al.,* 2011; Ferraz *et al.,* 2013; Fry *et al.,* 2011; Pan-ngum *et al.,* 2013). Generally, the better results of strips for NS1 detection were specific to primary infections (Nga *et al.,* 2007; Moorthy *et al.,* 2009; Tricou *et al.,* 2010; Hang *et al.,* 2009). But several scientists also mentioned better diagnostic accuracy for dengue NS1 antigen during the secondary infections (Najioullah *et al.,* 2011). NS1 antigen detection is very important for point of care diagnosis in the dengue endemic regions and it is found to be more useful for early phase of infection. Therefore, combination of NS1 antigen and IgM/IgG antibodies ICTs developed by different commercial companies appear to be more valuable for attaining the high sensitivity and specificity results. Recently, commercial kits for analyte combination of NS1 antigen and IgM/IgG antibodies developed by same companies are also evaluated by different researchers as mentioned in table 1. The overall sensitivities observed for ICTs for combination of analytes ranged from 80-93%. This new generation of ICTs is more efficient and showed higher sensitivities and specificities as compared to ELISA format tests (Lima *et al.,* 2010).

Urgent need for highly specific antibodies

The requirement for point of care diagnostic tests has directed the proliferation of antibody based RDTs for tropical diseases (dengue, malaria and leptospirosis) using the immunochromatographic test format (Blacksell, 2012). Highly specific MAbs can be produced in unlimited quantities against any target antigen. MAbs recognize unique antigenic epitopes, thus allowing the accurate diagnosis of infection (Siddiqui, 2010). The efficiency of RDTs/ICTs becomes higher with the use of more serotype specific monoclonal antibodies during the preparation of rapid diagnostic kits. The production of monoclonal antibodies for dengue virus NS1 antigen detection or combination of NS1/IgM/IgG detection was found to be significant, which led to the improvement of rapid diagnostic tests for dengue infections with higher sensitivity and specificity. The immunodiagnostic assays with high sensitivity for NS1 detection can also be developed by the use of bi-specific monoclonal antibodies which are engineered bi-functional proteins with dual binding properties within a single entity. The inexpensive and sensitive NS1 detection based immunoassay utilizing bi-specific antibodies for the diagnosis of dengue is recently developed by Ganguly *et al.* (2013). Bi-specific antibodies which are composed of fragments from two different monoclonal antibodies, have ability to increase the sensitivity of existing diagnostic assays due to the monovalency for antigen as well as direct binding to the enzyme (Malabadi *et al.,* 2012).

In this review, promising outcomes for economical and rapid immunochromatographic tests have been reported as compared to the existing laboratory techniques (table 1) but still a lot of scientific work has to be accomplished to take the technology to the next level. It is an urgent need to overcome the shortcomings for the improvement of existing technologies and instantaneous persuasion of new technologies for the proper control of dengue infections.

CONCLUSION

Dengue disease is spreading at alarming rate in almost every country located in the tropics. There is no suitable vaccine available in market until now. So, improved diagnostics is the only way for early and proper treatment of dengue infected patients. Although laboratory techniques like virus isolation in mosquito cell lines, PCR, IgM or NS1 capture ELISA have major advances in dengue diagnosis. However, all these techniques are expensive and require experienced technicians. In addition, most of dengue endemic developing countries do not have laboratory or cold storage facilities. These techniques cannot be implemented in the developing countries. Consequently, immunochromatographic assays based rapid diagnostic strips were found to be successful and efficient tools in such developing countries for point of care test. These strips are user friendly, cheap, and good sensitivity and specificity results can be obtained even at high temperature. The high efficiency of rapid diagnostic kits based on ELISA or immuno-chromatographic assays is depended on the use of highly specific antibodies during the preparation of kits. Accordingly, it is need of the hour to emphasize on the development of highly specific and more advance antibodies that can be more useful for the development of highly advance diagnostic assays.

REFERENCES

Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V and Flamand M (2002). Enzyme-linked immuno-sorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *J. Clin. Microbiol*., **40**(2): 376-381.

Alcon-Lepoder S, Sivard P, Drouet MT, Talarmin A, Rice C and Flamand M (2006). Secretion of flaviviral non-structural protein NS1: From diagnosis to pathogenesis. *Novartis Found Symp*., **277**: 233-247.

Andries A-C, Duong V, Ngan C, Ong S, Huy R, Srion KK, Te V, Bunthin Y, Try PL and Buchy P (2012). Field evaluation and impact on clinical management of a rapid diagnostic kit that detects dengue NS1, IgM and IgG. *PLoS Negl. Trop. Dis.*, **6**(12): e1993. Doi:10.1371/journal.pntd.0001993.

Barde PV, Godbole S, Bharti PK, Chand G, Agarwal M and Singh N (2012). Detection of dengue virus4 from central India. *Indian J. Med. Res.*, **136**(3): 491-494.

Bessoff K, Delorey M, Sun W and Hunsperge E (2008). Comparison of two commercially available dengue virus (DENV) NS1 capture enzyme-linked immunosorbent assays using a single clinical sample for diagnosis of acute DENV infection. *Clin. Vaccine Immunol*., **15**(10): 1513-1518.

Blacksell SD (2012). Commercial dengue rapid diagnostic tests for point-of-care application: Recent evaluations and future needs? *J. Biomed. Biotechnol.*, 151967.

Blacksell SD, Bell D, Kelley J, Mammen MP Jr., Gibbons RV, Jarman RG, Vaughn DW, Jenjaroen K, Nisalak A, Thongpaseuth S, Vongsouvath M, Davong V, Phouminh P, Phetsouvanh R, Day NP and Newton PN (2007). Prospective study to determine accuracy of rapid serological assays for diagnosis of acute dengue virus infection in Laos. *Clin. Vaccine Immunol.*, **14**(11): 1458-1464.

Blacksell SD, Jarman RG, Bailey MS, Tanganuchitcharnchai A, Jenjaroen K, Gibbons RV, Paris DH, Premaratna R, de Silva HJ, Lalloo DG and Day NP (2011). Evaluation of six commercial point-of-care tests for diagnosis of acute dengue infections: The need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. *Clin. Vaccine Immunol*., **18**(12): 2095-2101.

Blacksell SD, Jarman RG, Gibbons RV, Tanganuchitcharnchai A, Mammen MP Jr., Nisalak A, Kalayanarooj S, Bailey MS, Premaratna R, de Silva HJ, Day NP and Lalloo DG (2012). Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection. *Clin. Vaccine Immunol*., **19**(5): 804-810.

Blacksell SD, Newton PN, Bell D, Kelley J, Mammen MP Jr., Vaughn DW, Wuthiekanun V, Sungkakum A, Nisalak A and Day NP (2006). The comparative accuracy of 8 commercial rapid immuno chromatographic assays for the diagnosis of acute dengue virus infection. *Clin. Infect. Dis.,* **42**(8): 1127-1134.

Cahour A, Falgout B and Lai CJ (1992). Cleavage of the dengue virus polyprotein at the NS3/N84A and N84B/NS5 junction is mediated by viral proteinase N82B-N83, whereas NS4A/NS4B may be processed by a cellular protease. *J. Virol.,* **66**: 1525-1542.

Chaterji S, Allen JC Jr., Chow A, Leo YS and Ooi EE (2011). Evaluation of the NS1 rapid test and the WHO dengue classification schemes for use as bedside diagnosis of acute dengue fever in adults. *Am. J. Trop. Med. Hyg*., **84**(2): 224-228.

Chien LJ, Liao TL, Shu PY, Huang JH, Gubler DJ and Chang GJ (2006). Development of real-time reverse transcriptase PCR assays to detect and serotype dengue viruses. *Microbiol*., **44**(4): 1295-1304.

Chua KB, Mustafa B, Abdul-Wahab AH, Chem YK, Khairul AH, Kumarasamy V, Mariam M, Nurhasmimi H and Abdul-Rasid K (2011). A comparative evaluation of dengue diagnostic tests based on single-acute serum samples for laboratory confirmation of acute dengue. *Malays. J. Pathol*., **33**(1): 13-20.

Clarke DH and Casals J (1958). Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. *Am. J. Trop. Med. Hyg.,* **7**: 561-573.

Ferraz FO, Bomfim MRQ, Totola AH, Avila TV, Cisalpino D, Pessanha JE, da Glória de Souza D, Teixeira Júnior AL, Nogueira ML, Bruna-Romero O and Teixeira MM (2013). Evaluation of laboratory tests for dengue diagnosis in clinical specimens from consecutive patients with suspected dengue in Belo Horizonte. Brazil. *J. Clin. Virol.,* **58**(1): 41-46.

Filomatori CV, Lodeiro MF, Alvarez DE, Samsa MM, Pietrasanta L and Gamarnik AV (2006). A 5' RNA element promotes dengue virus RNA synthesis on a circular genome. *Genes Dev*., **20**: 2238-2249.

Fry SR, Meyer M, Semple MG, Simmons CP, Sekaran SD, Huang JX, Mcelnea C, Huang CY, Valks A, Young PR and Cooper MA (2011). The diagnostic sensitivity of dengue Rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Negl. Trop. Dis.,* **5**(6): e1199.

Ganguly A, Malabadi RB, Loebenberg R, Suresh MR and Sunwoo HH (2013). Dengue diagnosis: Current scenario. *Res. Biotechnol.,* **4**(2): 19-25.

Groen J, Koraka P, Velzing J, Copra C and Osterhaus AD (2000). Evaluation of six immunoassays for detection of dengue virus-specific immunoglobulin M and G antibodies. *Clin. Diagn. Lab. Immunol*., **7**(6): 867-871.

Gubler DJ (1999). Dengue viruses. *In*: GranoffA, Webster RG editors. Encyclopaedia of Virology 2™* ed., Academic Press, San Diego, pp.375-384.

Gubler DJ and Clark GG (1995). Dengue/dengue hemorrhagic fever: The emergence of a global health problem. *Emerg. Infect. Dis.,* **1**: 55-57.

Gurukumar KR, Priyadarshini D, Patil JA, Bhagat A, Singh A, Shah PS and Cecilia D (2009). Development of real-time PCR for detection and quantitation of dengue viruses. *Virol. J.,* **6**:10-17.

Halstead SB (1992). Dengue viruses. *In*: GorbachSl, Bartlett JG, Blacklow MR editors. Infectious Diseases, Philadelphia, Saunders, pp.281-304.

Hang VT, Nguyet NM, Trung DT, Tricou V, Yoksan S, Dung NM, Van Ngoc T, Hien TT, Farrar J, Wills B and Simmons CP (2009). Diagnostic accuracy of NS1 ELISA and lateral flow rapid tests for dengue sensitivity, specificity and relationship to viraemia and antibody responses. *PLoS Negl. Trop. Dis.,* **3**(1): e360.

Hidari KI and Suzuki T (2011). Dengue virus receptor. *Trop. Med. Health*, **39**: 37-43.

Hue KD, Tuan TV, Thi HT, Bich CT, Anh HH, Wills BA and Simmons CP (2011). Validation of an internally controlled one-step real-time multiplex RT-PCR assay for the detection and quantitation of dengue virus RNA in plasma. *J. Virol. Methods*., **177**(2): 168-173.

Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA,Pelegrino JL, Vázquez S, Artsob H, Drebot M, Gubler DJ, Halstead SB, Guzmán MG, Margolis HS, Nathanson CM, Rizzo-Lic NR, Bessoff KE, Kliks S and Peeling RW (2009). Evaluation of commercially available anti-dengue virus immunoglobulin M tests. *Emerging Infect. Dis.,* **15**(3): 436–440.

Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P and Hoke CH (1989). An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am. J. Trop. Med. Hyg.,* **40**: 418-442.

Jarman RG, Nisalak A, Anderson KB, Klungthong C, Thaisomboonsuk B, Kaneechit W, Kalayanarooj S and Gibbons RV (2011). Factors influencing dengue virus isolation by C6/36 cell culture and mosquito inoculation of nested PCR-positive clinical samples. *Am. J. Trop. Med. Hyg.,* **84**(2): 218-223.

Kimura R and Hotta S (1943). Experimental inoculation of dengue virus into mice. *Nippon Igaku.,* **3344**: 1378-1379.

Klungthong C, Gibbons RV, Thaisomboonsuk B, Nisalak A, Kalayanarooj S, Thirawuth V, Nutkumhang N, Mammen MP and Jarman RG (2007). Dengue virus detection using whole blood for reverse transcriptase PCR and virus isolation. *J. Clin. Microbiol.,* **45**(8): 2480-2485.

Kuberski TT and Rosen L (1977). Identification of dengue viruses using complement-fixing antigen produced in mosquitoes. *Am. J. Trop. Med. Hyg.,* **26**(3): 538-543.

Kumarasamy V, Wahab AH, Chua SK, Hassan Z, Chem YK, Mohamad M and Chua KB (2007). Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *J. Virol. Methods.,* **140**(1-2): 75-79.

Lai YL, Chung YK, Tan HC, Yap HF, Yap G, Ooi EE and Ng LC (2007). Cost-effective real-time reverse transcriptase PCR (RT-PCR) to Dengue virus followed by rapid smultiplex RT-PCR for serotyping of the virus. *J. Clin. Microbiol.,* **45**(3): 935-941.

Lam SK (1993). Rapid dengue diagnosis and interpretation. *Malays. J. Pathol.,* **15**: 9-12.

Lam SK (1995). Application of rapid laboratory diagnosis in dengue control. *Asia Pac. J. Mol. Biol. Biotechnol.,* **3**: 351-355.

Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ and Vorndam AV (1992). Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J. Clin. Microbiol.,* **30**: 545-551.

Lee YR, Huang KJ, Lei HY, Chen SH, Lin YS, Yeh TM and Liu HS (2005). Suckling mice were used to detect infectious dengue-2 viruses by intracerebral injection of the full-length RNA transcript. *Intervirology,* **48**(2-3): 161-166.

Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, Vaughn DW, Nisalak A, Ennis FA and Rothman AL (2002). High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J. Infect. Dis.,* **186**: 1165-1168.

Lima MRQ, Nogueira RMR, Schatzmayr HG and dos Santos FB (2010). Comparison of three commercially available dengue NS1 antigen capture assays for acute diagnosis of Dengue in Brazil. *PLoS Negl. Trop. Dis.,* **4**(7): e738.

Lindenbach BD and Rice CM (1997). Trans-Complementation of yellow fever virus NS1reveals a role in early RNA replication. *J. Virol.,* **71**: 9608-9617.

Mackenzie J, Jones M and Young PR (1996). Immunolocalization of the dengue virus nonstructural glycoprotein NS1 suggests a role in viral RNA replication. *J. Virol. Methods.,* **220**: 232-240.

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

Martinez E (1992). Dengue hemorragico en criancas. *In*: Marti J, Habana L editors. pp.1-180.

Matsumura T, Stollar V and Schlesinger RW (1971). Studies on the nature of dengue viruses. V. Structure and development of dengue virus in Vero cells. *Virol.,* **46**(2): 344-355.

Meiklejohn G, England B and Lennette EH (1952). Propagation of dengue virus strains in unweaned mice. *Am. J. Trop. Med. Hyg.,***1**(1): 51-58.

Monath TP, Wands JR, Hill LJ, Gentry MK, Gubler DJ, Monath TP, Wands JR, Hill LJ,Gentry MK and Gubler DJ (1986). Multisite monoclonal immunoassay for dengue viruses: Detection of viraemic human sera and interference by heterologous antibody. *J. Gen. Virol.,* **67**: 639-650.

Moorthy M, Chandy S, Selvaraj K and Abraham AM (2009). Evaluation of a rapid immuno chromatographic device for the detection of IgM&IgG antibodies to Dengue viruses (DENV) in a tertiary care hospital in South India. *Indian J. Med. Microbiol.,* **27**(3): 254-256.

Muylaert IR, Galler R and Rice CM (1997). Genetic analysis of the yellow fever virus NS1 protein: Identification of a temperature-sensitive mutation, which blocks RNA accumulation. *J. Virol.,* **71**: 291-298.

Najioullah F, Combet E, Paturel L, Martial J, Koulmann L, Thomas L, Hatchuel Y, Cabié A and Cesaire R (2011). Prospective evaluation of nonstructural 1 enzyme-linked immunosorbent assay and rapid immuno chromatographic tests to detect dengue virus in patients with acute febrile illness. *Diagn. Microbiol. Infect. Dis*., **69**(2): 172-178.

Nakamura H and Karube I (2003). Current research activity in biosensors. *Anal. Bioanal. Chem.,* **377**: 446-468.

Nawa M, Takasaki T, Yamada KI, Akatsuka T and Kurane I (2001). Development of dengue IgM-capture enzyme-linked immunosorbent assay with higher sensitivity using monoclonal detection antibody. *J. Virol. Methods.,* **92**: 65-70.

Nga TT, Thai KTD, Phuong HL, Giao PT, Hung LQ, Binh TQ, Mai VT, Van Nam N and de Vries PJ (2007). Evaluation of two rapid immunochromatographic assays for diagnosis of dengue among Vietnamese febrile patients. *Clin. Vaccine Immunol.*,**14**(6):799-801.

Nimmannitya S (1987). Clinical spectrum and management of dengue haemorrhagic fever. *Southeast Asian J. Trop. Med. Pub.Health.,* **20**: 325-330.

Oliveira De Paula S, Malta Lima D, Clotteau M, PiresNeto Rd Rda J and Lopes da Fonseca BA (2003). Improved detection of dengue-1 virus from IgM-positive serum samples using C6/36 cell cultures in association with RT-PCR. *Intervirology*., **46**: 227-231.

Osorio L, Ramirez M, Bonelo A, Villar LA and Parra B (2010). Comparison of the diagnostic accuracy of commercial NS1based diagnostic tests for early dengue infection. *Virology J.,* **7**: 361-370.

Pan American Health Organization (1994). Dengue and dengue hemorrhagic fever in the Americas. *Guidelines for Prevention and Control.* Scientific Publication No. 548.

Pan-Ngum W, Blacksell SD, Lubell Y, Pukrittayakamee S, Bailey MS, de Silva HJ, Lalloo DG, Day NP, White LJ and Limmathurotsakul D (2013). Estimating the true accuracy of diagnostic tests for dengue using bayesian latent class Models. *PLoS ONE*, **8**(1): e50765.

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, Margolis HS, Nathanson CM, Nguyen VC, Rizzo N, Vazquez S and Yoksan S (2010). Evaluation of diagnostic tests: dengue. *Nat. Rev. Microbiol.*, S30-S37.

Pelegrino JL, Arteaga E, Rodríguez AJ, González E, Frontela MD and Guzmán MG (1997). Standardization of immunohistochemical techniques for detecting dengue virus antigens in paraffin-embedded tissues. *Rev. Cubana. Med. Trop.,* **49**: 100-107.

Pok KY, Lai YL, Sng J and Ng LC (2010). Evaluation of nonstructural 1 antigen assays for the diagnosis and surveillance of dengue in Singapore. *Vector. Borne. Zoonotic. Dis.*, **10**(10): 1009-1016.

Puttikhunt C, Prommool T, U-Thainual N, Ong-Ajchaowlerd P, Yoosook K, Tawilert C, Duangchinda T, Jairangsri A, Tangthawornchaikul N, Malasit P and Kasinrerk W (2011). The development of a novel serotyping-NS1-ELISA to identify serotypes of dengue virus. *J. Clin. Virol.*, **50**(4): 314-319.

Raengsakulrach B, Nisalak A, Maneekarn N, Yenchitsomanus PT, Limsomwong C, Jairungsri A, Thirawuth V, Green S, Kalayanarooj S, Suntayakorn S, Sittisombut N, Malasit P and Vaughn D (2002). Comparison of four reverse transcription-polymerase chain reaction procedures for the detection of dengue virus in clinical specimens. *J. Virol. Methods.,* **105**(2): 219-232.

Rice CM, Lenches EM, Eddy SR, Shin SJ, Sheets RL and Strauss JH (1985). Nucleotide sequence of yellow fever virus: Implications for flavivirus gene expression and evolution. *Science*, **229**(4715): 726-733.

Rosen Land Gubler D (1974).The use of mosquitoes to detect and propagate dengue viruses. *Am. J. Trop. Med. Hyg*., **23**(6): 1153-1160.

Sang CT, Hoon LS, Cuzzubbo A and Devine P (1998). Clinical evaluation of a rapid immunochromatographic test for the diagnosis of dengue virus infection. *Clin. Diagnostic Lab. Immunol*., **5**: 407-409.

Shu PY, Chang SF, Kuo YC, Yueh YY, Chien LJ, Sue CL, Lin TH and Huang JH (2003). Development of group- and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus. *J. Clin. Microbiol.*, **41**: 2408-2416.

Siddiqui MZ (2010). Monoclonal antibodies as diagnostics; an appraisal. *Indian J. Pharm. Sci.,* **72**(1): 12-17.

Tang KF and Ooi EE (2012). Diagnosis of dengue: An update. *Expert. Rev. Anti. Infect. Ther.*,**10**(8): 895-907.

Tesh RB (1979). A method for the isolation and identification of dengue viruses, using mosquito cell cultures. *Am. J. Trop. Med. Hyg.,* **28**(6): 1053-1059.

Tontulawat P, Pongsiri P, Thongmee C, Theamboonlers A, Kamolvarin N and Poovorawan Y (2011). Evaluation of rapid immuno chromatographic NS1 test, anti-dengue IgM test, semi-nested PCR and IgM ELISA for detection of dengue virus. *Southeast Asian J. Trop. Med. Public Health*., **42**(3): 570-578.

Tricou V, Vu HT, Quynh NV, Nguyen CV, Tran HT, Farrar J, Wills B and Simmons CP (2010). Comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses. *BMC Infect. Dis.*, **10**: 142-149.

Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA and Nisalak A (2000). Dengue viremia titer, antibody response pattern and virus serotype correlate with disease severity. *J. Infect. Dis.*, **181**: 2-9.

Vaughn DW, Nisalak A, Kalayanarooj S, Solomon T, Dung NM, Cuzzubbo A and Devine PL (1998). Evaluation of a Rapid Immunochromatographic Test for diagnosis of dengue virus infection. *Clin. Microbiol.*, **36**(1): 234-238.

Vázquez S, Lemos G, PupoM, Ganzón O, Palenzuela D, Indart A and Guzmán MG (2003). Diagnosis of dengue virus infection by the visual and simple AuBioDot immunoglobulin M Capture System. *Clin. Diagn. Lab. Immunol*., **10**: 1074-1077.

Vázquez S, Sáenz E, Huelva G, González A, Kourí G and Guzmán M (1998). Detection of IgM against the dengue++ virus in whole blood absorbed on filter paper. *Rev. Panam. Salud. Publica.,* **3**: 174-178.

Waggoner JJ, Abeynayake J, Sahoo MK, Gresh L, Tellez Y, Gonzalez K, Ballesteros G, Pierro AM, Gaibani P, Guo FP, Sambri V, Balmaseda A, Karunaratne K, Harris E and Pinsky BA (2013). Single-reaction, multiplex, real-time RT-PCR for the detection, quantitation, and serotyping of dengue viruses. *PLoS Negl. Trop. Dis.*, **7**(4): e2116.

Wang SM and Sekaran SD (2010). Evaluation of a commercial SD dengue virus NS1 antigen capture enzyme-linked immunosorbent assay kit for early diagnosis of dengue virus infection. *J. Clin. Microbiol.*, **48**(8): 2793-2797.

Welsch S, Miller S, Romero-Brey I, Merz A, Bleck CK, Walther P, Fuller SD, Antony C, Krijnse-Locker J and Bartenschlager R (2009). Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell. Host. Microbe.*, **5**: 365-375.

Westaway EG, Mackenzie JM, Kenney MT, Jones MK and Khromykh AA (1997). Ultrastructure of Kunjin virus-infected cells: Colocalization of NS1 and NS3

with double-stranded RNA and of NS2B with NS3, in virus-induced membrane structures. *J. Virol.,***71**: 6650-6661.

World Health Organization (1986). Dengue hemorrhagic fever: Diagnosis, treatment and control. 2nd edition, *WHO*, Geneva. ISBN 9241545003.

World Health Organization (2012).Global strategy for dengue prevention and control 2012-2020.*WHO report*, Geneva. ISBN 789241504034.

Wu SJ, Lee EM, Putvatana R, Shurtliff RN, Porter KR, Suharyono W, Watts DM, King CC, Murphy GS, Hayes CG and Romano JW (2001). Detection of dengue viral RNA using a nucleic acid sequence-based amplification assay. *J. Clin. Microbiol.,* **39**(8): 2794-2798.

Young PR, Hilditch PA, Bletchly C and Halloran W (2000). An antigen capture enzyme linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. *J. Clin. Microbiol.,* **38**: 1053-1057.

Yuill TM, Sukhavachana P, Nisalak A and Russell PK (1968). Dengue-virus recovery by direct and delayed plaques in LLC-MK2 cells. *Am. J. Trop. Med. Hyg.*, **17**(3): 441-448.