# Laboratory Diagnosis of Dengue: A Review

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**ABSTRACT**

Dengue is an arthropod borne disease that has become important worldwide. There is still no specific drug available for treatment and also no protective vaccine that can be used. As such, specific diagnosis is essential to enable good management and prevention of large outbreaks. Diagnosis today in many countries is still based on serology though the detection of NS1 has slowly become incorporated. Diagnosis is critical for early intervention with specific preventive health measures to prevent fatalities and also to curtail spread and reduce economic losses. Serological assays mainly detect IgM which now as a single test is invalid unless a second sample is taken to confirm. As such to effectively diagnose dengue at all stages of infection, assays with two or more markers are required or two samples taken a few days apart. Other commonly used tests include NS1 detection, nucleic acid amplification and IgG detection. However the sensitivities of the current commercial kits vary quite considerably and have to be interpreted with caution. Hence knowledge of this disease is essential when conducting diagnostics for dengue.

**KEYWORDS:** Dengue, laboratory test, diagnosis, assay

**INTRODUCTION**

Dengue, an arthropod-borne disease is a major health concern especially in the tropics and subtropics.[1] Since the 1960s, incidences around the world have increased by more than 30-fold, with more than 100 countries in all continents being affected. It is estimated that approximately 390 million dengue infections occur yearly.[2] In Malaysia[3,4] as seen in Table I and Figure 1 the incidence has been rising in the last 15 years except for 2011 and 2012 where the incidence dropped but again rose that the following year. What is evident is that more and more reported cases are being confirmed by laboratory tests. Despite being around for centuries, dengue has neither the commercialized vaccine nor anti-dengue drugs mainly because the vaccine needs to be able to protect against all four DENV serotypes,[2] knowledge on the protective immune correlates is lacking,[3] there is absence of reliable animal models to represent dengue and controversial and limited understanding of dengue pathogenesis.[4] The four dengue serotypes can cause illnesses in humans traditionally as ranging from the self-limiting to the life-threatening dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS).[1] However, in 2009 WHO has suggested a revised classification and that is dengue with or without warning signs and severe dengue.[1,5] Classical dengue fever (DF) is generally self-limiting and is characterized by fever and a variety of non-specific signs and symptoms that makes it indistinguishable from other febrile illnesses. DHF is distinguished from DF by the onset of plasma leakage, marked thrombocytopenia, and a bleeding diathesis that can lead to shock.[6,7] Complications may occur, where patients may have liver failure and hypotension and if not clinically well-managed, may either succumb or recover fully at the convalescence stage. A clinical diagnosis is not always reliable as dengue has no pathognomonic clinical features that reliably distinguish it early from several other febrile illnesses as well as other closely related flaviviruses.[8,9] Hence, a diagnostic test needs to be conducted in order to confirm the diagnosis which will then allow proper management and treatment of the disease. When dengue virus invades the human body, the main defense is the immune system which comprises of an innate arm which responds immediately and an adaptive arm that specifically and efficiently targets the virus. The innate arm does not provide long term protection while the adaptive though taking longer to respond is said to provide immunity for life and is largely made up of antibodies. However in the early stages before the onset of antibodies diagnosis usually depends on detection/isolation of the virus/viral antigens. Dengue diagnosis is not only important for clinical management of patients, but also for intervention during outbreaks, epidemiological surveillance and for vaccine development and monitoring. Laboratory confirmation has become an essential part of diagnosing dengue. The main hurdle in developing an ideal diagnostic assay lies in the incompletely understood pathogenesis of dengue and also that multiple sequential infections occurs in dengue endemic areas. To further complicate this, when one is infected, he/she develops full immunity towards the particular infecting serotype and not towards the other 3 serotypes. Around the world vigorous and intensive research efforts are being carried out but, the understanding of dengue pathogenesis still remains obscure and controversial. Most of the theories (antibody dependent enhancement, cross-reactive cellular responses, and original antigenic sin) revolve around secondary infections with a different serotype than that of the first.[10] Nonetheless, primary and secondary infection statuses play important roles

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in dengue diagnosis. Therefore for a diagnostic assay to be useful and effective, it is essential for users to have some degree of confidence in the test in order to improve disease management, especially in the acute early stage and for detecting signs of severity. In the absence of a vaccine and antiviral therapies for dengue, early diagnosis is important for timely clinical intervention, etiological investigation and for disease control.8 With the possible introduction of vaccine in the near future, dengue diagnosis will become even more important, as data from vaccine efficacy trials will determine the usefulness of candidate vaccines.

An ideal dengue diagnostics would be rapid, simple, with high sensitivity and specificity, preferably able to differentiate between primary and secondary infections, as well as to serotype the viruses and most important of all be affordable. The optimal time frame for diagnosis would be from the onset of dengue symptoms to 10 days post-infection.[11,12] Nevertheless, not all are able to be diagnosed within this time frame.[1] Some people consult the physician only when in dire situations,[2] many people in third world countries rely heavily on traditional healing,[3] 2% of world population do not seroconvert[4] and there is a high number of dengue asymptomatic cases.[9] Therefore, this ideal diagnostic test should be able to detect genuine dengue cases at any stage of illness. As the current diagnostic tools which are mainly either serologically based, nucleic acid-based and antigen detection, a good understanding of clinical conditions of dengue patients is essential for appropriate usage of these tests. Dengue diagnosis is divided into two main phases, the early phase and the late phase. In the early phase, the approach of diagnosis is via viral detection, viral RT-PCRs, and antigen detection. Meanwhile, the later phase of diagnosis is mainly through serological testing. Despite the many efforts to create a single assay that could confirm dengue, that goal has not been reached. Nevertheless, many researchers around the world are still attempting to develop a more efficient and reliable diagnostic method.

Upon infection with dengue virus the first class of antibodies to be produced is IgM and can be detected from day 3 onwards and by day 10 of illness, at least 99% of dengue patients would have mounted IgM levels.[1] This class of antibody however persists in the body for up to 3 months1,10 and as a result confirmation requires a second sample to be done as few days apart. In secondary infections, IgM is still mounted but at lower levels and because of the presence of memory IgG antibodies may sometimes not be detected.[11,13] This is because it is produced almost simultaneously and affects the detection of IgM as memory specific IgG tends to overwhelm as well as block detection of IgM. When the different serotypes of dengue co-exist and multiple infections occur, this causes complications in the serological diagnosis of this disease, as a result of pre-existing antibodies, and also because these antibodies have lower affinity against the second infecting serotype.

Hence this has prompted many investigators to develop diagnostics for the detection of viral RNA and viral proteins. With the advent of the polymerase chain reaction (PCR) techniques, many in-house assays and commercial kits have been developed and further simplified to enable earlier detection of the virus. Most RT-PCR developed are serotype-specific, or genus-specific,[14] and many have also developed real time assays to detect all four serotypes simultaneously.[14,15] Apart from this the viral non-structural protein-1 (NS1) was shown to be secreted in a large number of patients in the early phase of infection and an enzyme-linked immunosorbent assay (ELISA) was developed to detect this viral protein (Table 2). This assay has become a rapid, sensitive, specific test and is relatively inexpensive as compared to molecular diagnostics assays.

**Table 1**. List of dengue cases reported in Malaysia from year 2001 to 2015

| Year | Dengue cases | Dengue with DF/DHF* | Deaths |
|---|---|---|---|
| 2000 | 7103 | 5500 | 41 |
| 2001 | 16,368 | 11,000 | 45 |
| 2002 | 32,767 | 25,000 | 100 |
| 2003 | 31,545 | 29,000 | 60 |
| 2004 | 33,895 | 31,000 | 95 |
| 2005 | 39,654 | 33,000 | 100 |
| 2006 | 38,556 | 30,000 | 85 |
| 2007 | 48,846 | 42,000 | 90 |
| 2008 | 49,335 | 44,000 | 100 |
| 2009 | 41,486 | 39,000 | 78 |
| 2010 | 46,171 | 44,000 | 121 |
| 2011 | 19,884 | 19,000 | 45 |
| 2012 | 21900 | 19,500 | 100 |
| 2013 | 43346 | 40,000 | 120 |
| 2014 | 76079 | 72,300 | 176 |
| 2015 | 42,645 | 40,000 | 272 |

*Cases confirmed by laboratory tests

**Table 2.** List of Dengue PCR assays developed and evaluated

| Virus Strain | PCR method | Sensitivity | Specificity | Number of Sample | Target region | Reference |
|---|---|---|---|---|---|---|
| DENV 1,2,3,4; JE; YF | RT- PCR 1 step | NA | NA | NA | NS5/3'NC | Tanaka 1993 |
| DENV 1,2,3,4; JE;YF | RT- PCR 1 step | 100%/ Specificity NA | NA | 115 serum | NS5 | Chang et al., 1994 |
| DENV1,2,3,4; JE; W; YF | RT-PCR 2 step | 99% | 66% | NA | NS5/ 3'NC | Pierre et al., 1994 |
| DENV 1,2,3,4; JE; POW | RT – PCR 2, semi nested | 100% | NA | 130 serum | NS1 | Meiyu et al., 1997 |
| All 66 viruses | RT-PCR 1 step | 100% | 20% | NA | NS5 | Kuno 1998 |
| DENV 1,2,3,4 | Real time Taq Man | 80% | 90% | 25 | NS5 | Laue et al., 1999 |
| DENV 1,2,3,4 | NASBA | 100% | 94.5% | 67 | 5' UTR | Wu et al., 2001 |
| DENV 1,2,3,4 | Real Time 2-step Taq Man | 92.8 | 92.4% | 4 | 3'NC | Houng et al., 2001 |
| DENV 1,2,3,4 | NASBA | NA | NA | 34 | 5' UTR | Usawattanakul et al., 2002 |
| DENV 1,2,3,4; WN; YF | RT- PCr 2; Seminested | 90 %/ Specificity NA | NA | Virus stock 1 CSF | NS5 | Scaramozzino et al., 2002 |
| DENV 1,2,3,4 | RT-PCR | 80 %/ Specificity NA. | NA | NA | NA | Lemmer et al., 2004 |
| DENV 1,2,3,4; JEV; WNV; St. Louis EV | LAMP PCR | 100% | NA | 100 | 3' NCR | Parida et al., 2005 |
| DENV 1,2,3,4 | SYBR Green 1 Real Time | 97.4% | NA | 39 | 3 ' NTR | Chutinimitkul et al., 2005 |
| DENV2; EV71 | Micro RT-PCR | 90% | NA | NA | 3'NC | Liao et al., 2005 |
| DENV 1,2,3,4 | Real Time Fourplex PCR | 100% | NA | 40 | CAP PrM | Johnson et al., 2005 |
| DENV 1,2,3,4 | Multiplex SYBR Green Real Time | 75% | 99% | 200 | PrM Capsid | Yong et al., 2006 |
| DENV 1,2,3,4; SLE; WNV | Real time PCR | 90% | D1-0.3; D2-0.3;D3-0.06; D4-2.6 | NA | NS5 | Ayers et al., 2006 |
| DENV 1,2,3,4 | RT-PCR 1 step | 100 %/ Specificity NA | NA | 376 | NS5 | Kong et al., 2006 |
| DENV 1,2,3,4; WN | Real time PCR | 100%/ NA | NA | NA | Capsid/ membrane/ NS5 | Dyer et al., 2007 |
| DENV 1,2,3,4 | Multiplex RT-PCR | 99% | 100% | 280 | NA | Yong et al., 2007 |
| NA | | 100 %/ Specificity | NA | 100 serum | NA | Dumoulin et al., 2008 |

| | | | | | | |
|---|---|---|---|---|---|---|
| DENV 3 | Real time Taq Man | 84% | 66% | NA | NA | Damodar et al., 2011 |
| DENV 1,2,3,4 | Single-reaction, multiplex, real-time rt-PCR | 97.2 % | 90.2% | 25/63 | Capsid/ Membrane | Waggoner et al., 2013 |
| NA | RT PCR 2 step | 100 % | NA | NA | NS1 | Sasmono et al., 2014 |
| DENV 1,2,3,4 | RT-PCR 1 step | 95.2% | D1-20% D2-50% D3-90% D4-90% | NA | NS1 | Kim et al., 2015 |
| DENV 4 | RT-PCR 1 step | 90% | 25.2 | 100 | NA | Waggoner et al., 2015 |

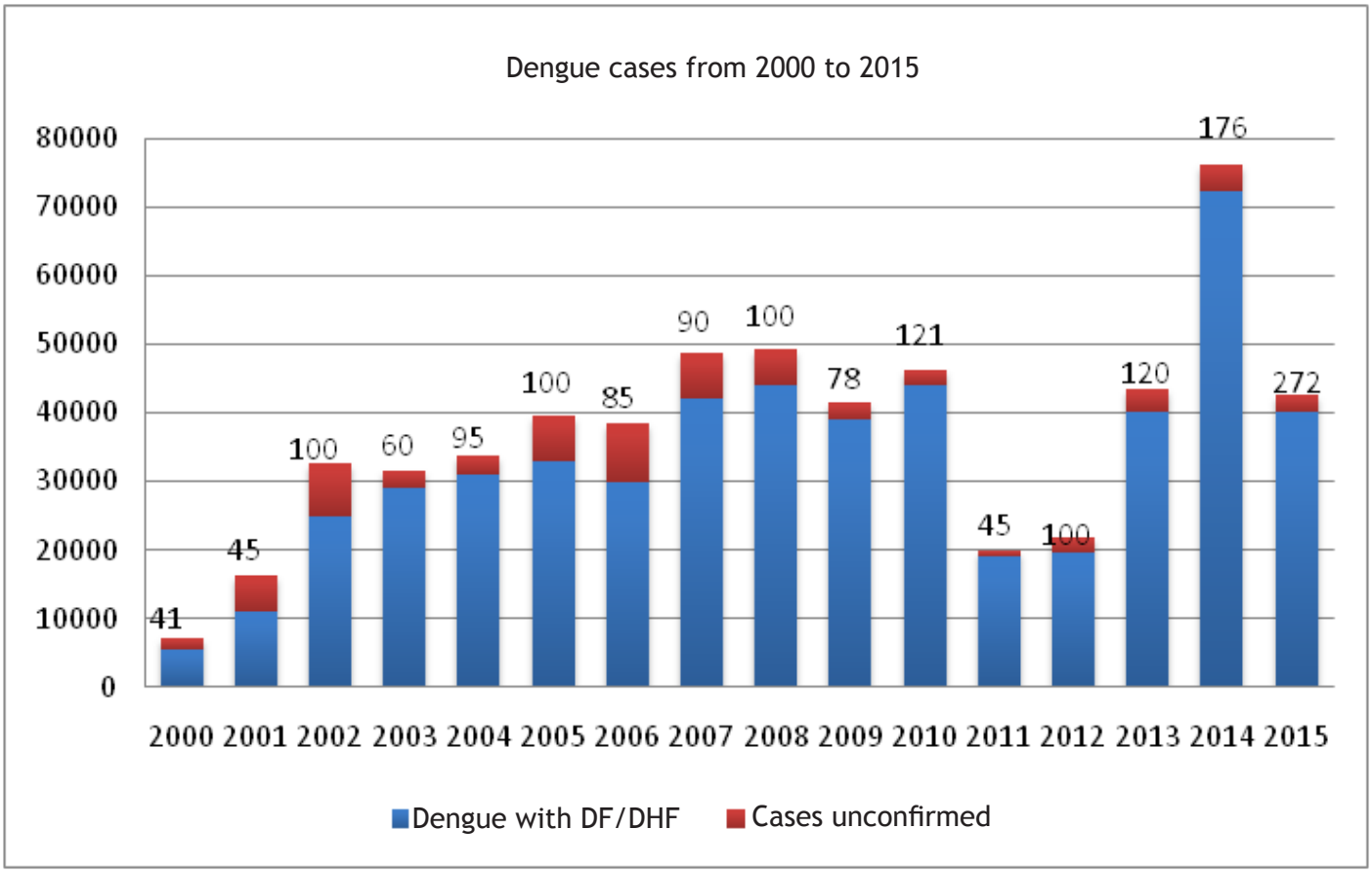


**Figure 1**: List of dengue cases reported in Malaysia from 2000 to 2015. The graph represents the total number of confirmed cases with dengue fever (DF) and dengue haemorhaghic fever (DHF). Each bar is differentiated into two colours based on confirmed dengue cases with DF/DHF and unconfirmed. The highest dengue confirmed cases are recorded in the year 2014 and lowest recorded in year 2000. The number of deaths recored are mentioned on the top of each bar respectively, with year 2000 being the lowest (40) and 2015 (until May 18, 2015) being the highest (272).

Diagnosis in the early phase of dengue infection

1. *Virus isolation*

Virus isolation has always been the gold standard for any viral disease. Cell lines used to grow the virus include mosquito cell lines (C6/36 and AP61) or mammalian cell lines (Vero, LLC-MK2 or BHK-21).[16,17] Virus can also be isolated using intracerebral inoculation of sucking mice. Traditionally 75ml flasks were used but we have now adapted to using 6-well microtitre plates thus bringing the time of harvest down from 7-12 days to 5-6 days and are able to do multiple samples simultaneously in a single plate. Sometimes more than 2 passages may be required to isolate the virus. Following harvest confirmation tests are carried out using dengue serotype specific fluorescent monoclonals in a UV microscope. A real time Taqman PCR can also

be used for confirmation. As such this method requires skill and substantial equipment to be carried out and is not the choice for many laboratories. This method depends heavily on survival of the sample. Hence this directly affects the time frame when the sample can be tested and also timely and proper storage of the samples are pertinent, as temperature may affect virus viability. However virus isolation remains very useful and relevant as a diagnostic tool, especially for monitoring of dengue epidemiology and evolution as well as determining its antigenic drift.

2. *Nucleic acid detection*

Viraemia and antigenemia is usually seen early in the course of a disease and as such specimens obtained during this time can confirm the infecting agent. This can be done using Polymerase Chain Reaction (PCR) or NS1 antigen assays. Early detection allows identification of pathogen, thus enabling initiation of appropriate clinical management and monitoring of patients for a lapse into more severe manifestations of dengue. PCR relies on amplifying DNA via thermal cycling using primers against target as template for replication. This has revolutionized diagnostics and has been used to diagnose many other bacterial infections as well as for leukaemia and many viral infections. For dengue however reverse transcriptase (RT) needs to be incorporated as the RNA has to be reverse transcribed into complementary DNA (cDNA) before amplification is carried out. PCR involves isolating the viral genome from most human samples (whole blood, serum, CSF, PBMCs, urine and autopsy tissues) and then amplifying them using protocols that are self-developed or using commercialised kits. The quality of nucleic acid extracted is important and there are many available kits that do this accurately and efficiently. This is an important crucial step before any PCRs are done, as what will be amplified depends on the quality of the extracted RNA reflects. Hence proper validation and standardization of this step is crucial in molecular diagnostics of dengue. Also of concern is the likelihood of cross-contamination as there are many pipetting steps involved. However a self-contained disposable cartridge microsystem was developed to overcome the problem. Over the years many in-house RT-PCRs for detection of dengue viruses have been developed (Table 2). Many of these RT-PCRs developed targeting different regions of the viral genome. Some detect the virus, while others detect serotype and quantitate. The real time RT-PCR uses either non-specific fluorescence dyes (i.e. SYBR green) that binds to any double stranded DNA or specific oligonucleotide probes with fluorescence reporter dye (i.e. TaqMan® Probes) that only allows detection when hybridized to specific DNA targets. The most popular ones use non-specific fluorescence dyes as they are least expensive with the major advantage being its ability to bind to any double-stranded (ds)-DNA which includes non-specific PCR products and primer dimers. This could lead to inaccurate quantification of intended target sequence and hence may cause an overestimation of DENV RNA concentration. Thus with this assay it is essential to stringently follow the various steps l the design of this assay. The greatest advantage of the real time assay is the ability to determine viral titre early in dengue illness, enabling physicians to take early course of action in managing the dengue patient. External quality assurance conducted on the many assays developed has shown that only 10.9% met all the criteria with optimal performance (sensitivity, specificity, serotyping and quantification) even though they had claimed enhanced analytical sensitivity and specificity in detecting dengue viruses. Also of concern is that there were different reproducibility rates though this may be due more to the skills of the individuals conducting the tests.[18,19] As seen in Table 2, the sensitivities vary from 75-99% and specificities from 20-100%. This also depends on the number of samples evaluated and the strain of viruses used in development. Not all developed assays evaluated specificity against related viruses.

False negatives/positives are also an issue indicating a need to improve specificity and/or to take precaution to avoid cross-contamination. Hence rigorous evaluation of available molecular diagnostics for dengue should be carried out in an organized and systematic manner to improve overall diagnostic performance. Other nucleic acid detection assays include the nucleic acid sequence-based amplification (NASBA),[20,21,22] the loop-mediated isothermal amplification (LAMP)[23, 24] and transcription-mediated amplification (TMA).[25] However none of these have been fully evaluated and validated even though they may be rapid, accurate and cost-effective.

3. *NS1 detection*

The 50kDa NS1 protein has become the most recent target in the development of dengue diagnostics. This protein is synthesized by all flaviviruses and has been shown to be secreted from infected cells at varying concentrations. Of late its presence in high concentrations in the plasma or serum may be indicative of impending risk of severe dengue. However NS1's role as a cause of plasma leakage or an effect of this leakage is yet to be ascertained.[26,27] Current commercial kits (Table 3) employ the ELISA method for the detection of this antigen. Most use a cocktail of monoclonal antibodies in order to ensure that NS1 from all serotypes are detected. Many of these commercialized kits can be used for diagnosis in the acute stage up to the defervescence stage (day 1-8 of fever onset) and have become useful especially in low resource settings and laboratories with limited skilled personnel. NSI detection however will decrease with the advent of both IgM and IgG antibodies. However, in some studies NS1 has been detected in convalescent sera up to day 18.[70,74] As seen in Table 4 the commercialized NS1 ELISAs and rapid diagnostics test have been evaluated in many dengue endemic countries [28-46] and each showed different sensitivities ranging from 45% to 91%. Specificity has also been shown to be problematic as cross reactions are noted with many other febrile illnesses.[47,48] NSI was also detected at higher levels in urine of DHF patients thus indicating a probable role in disease severity. This also has implications in terms of sample type which are not

invasive and does not require specialized equipment. It may be an alternative sample type when using the rapid tests for instance. However further studies are required to assess this aspect.[49,50] Developments have also begun to use this protein for serotyping and this might be useful in correlating further the infecting serotype with disease severity. Others have taken this further and were able to differentiate the four dengue serotypes from other flaviviruses.[24]

**Table 3:** List of Dengue NS1 antigen detection tests

| Test Name/ Company | Serotype | Sensitivity | Specificity | Significance | Reference |
|---|---|---|---|---|---|
| 1.PanBio NS1 ELISA | DENV(1-4) | 91.6% | 100% | n=206, P= 0.005, CI=95% | Sekaran SD et al., 2007 |
| 2. Platelia NS1antigen Biorad labs, France | DENV(1-4) | 68.1%( 61-71) | 96% ( 96-100) | n= 348, CI= 95% | Bessoff et al., 2008 |
| 3. Platelia NS1antigen | DENV(1-4) | 83.2% (77.5-100) | 100% (92.1-100) | n= 253, CI= 95% | Dussart et al., 2008 |
| 4. Platelia NS1antigen, Biorad Labs, France | DENV(1-4) | 66.3% | n/a | n=1284, P=0.05,CI=95% | Guzman et a., 2010 |
| 5. SD Duo NS1 | DENV(1-4) | 65.41%(58.4-72.32) | 98.7%(96.2-100) | n=320, CI= 95% | Wang et al., 2010 |
| 6. SD NS1 Ag ELISA | DENV(1-4) | 76.7% | 98.3% | n= 399, CI=95% | Wang et al., 2010 |
| 7. SD Bioline Dengue Diagnostics, Korea | DENV(1-4) | 44.8%(38-51) | 93.2%(88-87) | n= 210, P= 0.213, CI=95% | Blacksell et al., 2011 |
| 10. Venture labs, NS1 Ag capture | DENV(1-4) | 93.2% | n/a | n=116, CI=95% | Anders et al., 2012 |
| 11. Panbio NS1 Ag RDT | DENV(1-4) | 45.9%(40-51) | 97.9%(95-98) | n=549, CI=95% | Pan-ngum et al., 2013 |
| 12. NS1 antigen strip | DENV(1-4) | 54.8% (43.5-65.7) | 95.1% (92.7-96.8) | n=549, CI=95% | Pan-ngum et al., 2013 |
| 13. NS1 Ag RDT | DENV(1-4) | 88.9% (78.1-93.2) | 100%(72.5-100) | n=146, CI=95% | Ferraz et al., 2013 |
| 14. InBios NS1 kit InBios International, USA. | DENV(1-4) | 95% | 98.3% | n= 96, P=0.05, CI=95% | Aryati et al., 2010 |
| 15. SD NS1 ELISA | DENV(1-4) | 87.5% | 94.64% | n=397, P<0.05 ,CI=95% | Sánchez-Vargas et al., 2014 |
| 16. Pan-E Dengue Early ELISA. PanBio, Australia. | DENV(1-4) | 64.9% (58-72) | 97.8% (97-100) | n=150 , P=0.005, CI= 95% | Pal et al., 2014 |
| 17. Pan-E Dengue Early ELISA | DENV(1-4) | 66% | 100% | n=150 , P=0.005, CI= 95% | Shenoy et al., 2014 |
| 18. NS1 Dengue (ICT) | DENV(1-4) | 55.61% (50 to 71.9%) | 90% (91.59 to 100%) | n= 91, P= 0.005, CI= 95% | Naz et al., 2014 |
| 19. PanBio Dengue Duo Cassette | DENV(1-4) | 92.1%(87.8-95.8) | 62.2%(54.5-69.5) | n=135, CI=95% | Pal et al., 2015 |

$1^0$ & $2^0$ =primary & secondary; DENV= dengue virus; n= number of patients; CI= confidence interval; P= significance; RDT= rapid diagnosis test; ICT= Immuno-chromatographic test.

**Table 4:** List of commercialized IgM kits evaluated

| Test Name | Sample type | Sensitivity | Specificity% | Significance | Reference |
|---|---|---|---|---|---|
| PanBio IgM RDT | DENV(1-4) | 77.8% | 90.6% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| Pentax IgM RDT | DENV(1-4) | 97.7% | 76.6% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| SD Bioline IgM RDT | DENV(1-4) | 60.6% | 90.0% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| Zephyr IgM RDT | DENV(1-4) | 20.5% | 86.7% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| Focus IgM Capture ELISA | DENV(1-4) | 98.6% | 79.9% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| Omega Capture ELISA | DENV(1-4) | 62.3% | 97.8% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| Omega in-direct Capture ELISA | DENV(1-4) | 61.5% | 84.6% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| Panbio IgM Capture ELISA | DENV(1-4) | 99% | 84.4% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| SD Bioline Capture ELISA | DENV(1-4) | 97.6% | 86.6% | n=350, P<0.0001, | Hunsperger et al., 2009 |
| SD Duo IgM RDT | DENV(1-4) | 53.5% | 100% | n=320, CI= 95% | Wang et al., 2010 |
| SD Bioline Dengue Duo kit | DENV(1-4) | 96% | 98.4% | n=161, P=0.005, | Valdez et al., 2011 |
| Venture IgM capture ELISA | DENV(1-4) | 68.7% | 100% | n= 116, CI= 95% | Anders et al., 2012 |
| Venture IgM capture ELISA | DENV(1-4) | 100% | 75% | n=116, CI= 95% | Anders et al., 2012 |
| SD Bioline Dengue Duo kit | DENV(1-4) | 97.5% | 100% | n= 194, P= 0.178, CI= | Blacksell et al., 2012 |
| Pan-E Dengue Early ELISA | DENV(1-4) | 83.2% (78-87) | 87.8% (82-93) | n= 194, CI=95%, | Blacksell et al., 2012 |
| Panbio duo cassette IgM | DENV(1-2) | 61% (53.7-68.3) | 100% | n=172, P=0.05, | de la Cruz et al., 2012 |
| SD Bioline Dengue Duo kit | DENV(1-4) | 98.3% | 100% | n= 180, P=0.05, CI= 95% | Aryati et al., 2013 |
| Panbio duo cassette IgM | DENV(1-4) | 50%(38.9-61.1) | 89.5%(86.3-92.1) | n=549, CI= 95% | Pan-ngum et al., 2013 |
| IgM ICT | DENV(1-4) | 55.61%-71.9% | 91.59 to 100% | n= 91, P= 0.005, CI= 95% | Naz et al., 2014 |
| SD Bioline Dengue Duo kit | DENV(1-4) | 60.51% (53.40-67.63 | 94.06 (90.55-97.57) | n=397, P<0.05 ,CI=95% | Sánchez-Vargas et al., 2014 |
| PanBio Dengue IgM | DENV(1-4) | 77.8% | 90.6% | n=350, P<0.0001, CI=95% | De Decker e al., 2015 |

**Diagnosis in the later phase of dengue infection**

In the diagnosis of a viral infection viral isolation/ detection is the gold standard however it is more convenient and is usually the method of choice to detect antibodies. However to confirm the infection a paired sample must be collected and a four-fold rise in titre or an increase in intensity needs to be shown. The classes of antibodies chosen are usually IgM and IgG. Also it is important to note that antibodies are usually not detected till after the third day of onset of illness. The detection of IgM is indicative of a current infection while IgG may imply a recent or past infection. IgA is the other class of antibody that investigators have begun to use. In dengue the common serological assays include the hemagglutination inhibition test (HI), the plaque reduction neutralization test(NT), the IgM capture ELISA and the IgG capture ELISA. Major limitations of these assays are the need for paired samples thus taking more than a week to diagnose,[2] the presence of past IgM antibodies for 60-90 days,[3] high cross-reactivity with other flaviviruses or even with alphaviruses,8 the inability to determine the serotype9 and their detection only occurring at least 4-5 days post onset of illness thus making them not rapid. As such these tests if not done in pairs cannot provide a definite diagnosis and remain then presumptive.

The HI was the gold standard for many years and was very useful for seroepidemiological studies and for differentiating primary and secondary infections. The antibody titers of convalescent phase samples of a primary infection are usually below 1:640 while in a secondary or tertiary infection the titre is usually higher than 1:5120. However it does not allow discrimination between infections of other flaviviruses making it impractical to be used in countries where flavivirus infections are endemic. It is also a laborious test, requiring at least 3 days, easy access to goose blood and serum processing. The PRNT is most useful in a primary infection and is the most sensitive and specific serological assay as a relatively monotypic response is observed during the convalescent phase. However in areas where all dengue serotypes are prevalent, sequential infection is known to occur frequently and the neutralizing antibodies are cross reactive and not reliable in determining serotype. However the greatest disadvantages lie in the standardization in many aspects including cell lines, virus strains and concentration, incubation temperatures and time, rendering them tedious, labour-intensive, with constant amount of variation by different laboratories, and therefore, a less preferred method of diagnosis.

ELISA has become the most widely used serological method for dengue diagnosis. There is a reasonable amount of sensitivity and it is easy to perform requiring no sophisticated equipment. Early diagnosis is important and as the viremic phase is narrow and declines rapidly, the detection of antibodies such as IgM has become the preferred choice to diagnose dengue. The MAC-ELISA has since become the standard method for diagnosing dengue infected patients since anti-dengue IgM appears within five days of the first clinical symptoms. However the emergence varies from patient to patient from day 2 to day 9 in some cases. Also it cross-reacts with other flaviviruses such as Japanese encephalitis, St. Louis encephalitis and yellow fever viruses. Over the years, many laboratories have developed their own in-house MAC-ELISA51-53 and commercialized dengue IgM kits have been sprouting (Table 4) with most having variable sensitivity (61.5-99%) and specificity (79.9-97.8%). The rapid diagnostics tests, on the other hand, have lower sensitivities in those countries that conducted the evaluations with sensitivities ranging from 20.5-97.7% and specificities ranging 76.6 – 90.6%.48 The efficacy of IgM kits which comes in multiple formats (microplates, strips as well as cassettes) are most of the time not strictly evaluated with well referenced serum panels. The capture ELISA has the advantage of fast detection and is valuable when there are a large number of samples especially during epidemics. A limitation of the IgM ELISAs is that IgM persists for up to 3 months and a second confirmatory test must be carried out to ensure that what is detected is current and not a recent past infection.

Apart from IgM, other assays have been developed using similar formats to detect IgG and IgA antibodies and commercial kits developed (Table 5). IgG ELISAs are not very specific, cross-reacting with other flaviruses and not useful for dengue serotype identification. However they are useful for seroepidemiological studies. Recently, dengue specific IgA has become a target of interest in dengue diagnosis as the IgA has been shown to appear earlier than IgM and IgG and also decreases rapidly making it a better indicator of recent dengue. With this antibody it is possible to determine levels in saliva and hence making this a non-invasive test. Evaluations conducted (Table 6) indicate a sensitivity of 94.4% and a specificity of 74.7% in serum and slightly lower levels in saliva. Using a novel immunochromatographic test based on reverse flow technology[54,55] another IgA ELISA showed 99.4% sensitivity and 99.2% specificity, with comparable detection rates over day of illness with the RT-PCR, in the 179 tested samples.56 It could be used as a simple point of care device where facilities are minimal.

**Combination Assays**

The combined uses of antigen and antibody assays have overall resulted in increased ability to diagnose dengue. Using more than one marker has resulted in confirmation of cases especially where only one sample is obtained. As seen in Table 6 sensitivities range from 68.9 - 93.9% while specificities ranged from 72.5 - 98.75%. These kits are increasingly being used especially the rapid tests which allow diagnosis within 20 minutes with as little as 100ul of whole blood. These evaluations show that the combination kits are useful, sensitive, specific and rapid for early diagnosis of acute dengue infection.

**Table 5**: List of commercial IgG and Ig A kits developed and evaluated.

| Test Name | Sample type | Sensitivity | Specificity% | Significance | Reference |
|---|---|---|---|---|---|
| Venture labs IgG capture | DENV(1-4) | 91.9% | 92.3% | n=116, CI=95% | Anders et al., 2012 |
| Venture labs IgG capture | DENV(1-4) | 93% | 95.6% | n=116, CI=95% | Anders et al., 2012 |
| SD Bioline Dengue IgG | DENV(1-4) | 97.5% | 100% | n= 124 , P= 0.178, CI= 95% | Aryati et al., 2013 |
| Panbio IgG ELISA | DENV(1-4) | 39.8%(4-46) | 95.8% (91-98) | n= 250, CI=95% | Hasan et al., 2013 |
| Panbio duo cassette | DENV(1-4) | 62.1%(52.7-71.3) | 84.5%(80.6-88.1) | n=549, CI=95% | Pan-ngum et al., 2013 |
| Panbio Dengue IgG | DENV(1-4) | 91.8% (86.3-95.3) | 96% (86-95) | n=135, P<0.05 ,CI=95% | Gan et al., 2014 |
| SD Bioline IgG | DENV(1-4) | 90.06%(85.28-94.84) | 92.48%(88.82-96.14) | n=397, P<0.05 ,CI=95% | Sánchez-Vargas et al., 2014 |
| Panbio IgG capture ELISA | DENV(1-4) | 81.2%(76-86) | 63.5% (55-71) | n=195, CI= 95% | Pal et al., 2015 |
| IgA (AAC-ELISA) | DENV(1-4) | 61% | 54.5% (53.7-68.3) | n=172, P=0.05, CI=95% | Vazquez et al., 2007 |
| IgA (AAC-ELISA) | DENV(1-4) | 93% | 90% (87-96) | n=134, P=0.25, CI=95% | Vazquez et al., 2007 |
| Assure IgA RDT | DENV(1-4) | 99.4% | 100% | n= 178, CI=95% | Ahmed et al., (2010) |
| Assure IgA RDT | DENV(1-4) | 85.5% | 80% | n= 233, CI= 95% | Tan et al., (2011) |
| Assure IgA RDT | DENV(1-4) | 85.1% | 61% | n=172, CI=95% | de la Cruz Hernández et al., 2012 |
| ASSURE® Dengue IgA RDT | DENV (1,2) | 61% | 54% (53.7-68.3) | n=172, P=0.05, CI=95% | de la Cruz Hernández et al., 2012 |
| Platelia Dengue IgA Capture (Biorad) | DENV(1-4) | 93% | 88% (87-96) | n=134, P=0.25, CI=95% | De Decker et al., 2015 |

$1^0$& $2^0$ =primary & secondary; DENV= dengue virus; n= number of patients; CI= confidence interval; P= significance; RDT= rapid diagnosis test; ICT= Immuno-chromatographic test.

Table 6: List of Combination Tests (NS1+IgM+IgG)

| Test Name | Sample type | Sensitivity | Specificity% | Significance | Reference |
|---|---|---|---|---|---|
| SD Duo NS1 + IgM | DENV (1-4) | 88.65% | 98.75% | n=320, CI= 95% | Wang et al., 2010 |
| PanBio Dengue Early detection | DENV (1-4) | 68.9% | 96.7% | n= 298, CI= 95% | Fry et al., 2011 |
| Panbio NS1+ IgM | DENV (1-4) | 79.8 (69.6-87.7) | 86.2% (82.8-89.2) | n=549, CI=95% | Hasan et al., 2013 |
| Panbio NS1+IgM+IgG | DENV (1-4) | 92.9 (85.1-97.3) | 72.5% (68.2-76.5) | n=549, CI=95% | Hasan et al., 2013 |
| SD Bioline NS1+IgM+ IgG | DENV (1-4) | 90.65%(87.24-94.05) | 89.66%(82.68-96.63) | n=397, P<0.05 ,CI=95% | Sánchez-Vargas et al., 2014 |
| SD Dengue Duo NS1+IgM+IgG | DENV (1-4) | 93.9% | 92% | n=135, P<0.05 ,CI=95% | Gan et al., (2014) |
| SD Dengue Dengue Duo NS1+ IgM | DENV (1-4) | 91.8% | 96% | n=135, P<0.05, CI=95% | Gan et al., (2014) |

$1^0$ & $2^0$ =primary & secondary; DENV= dengue virus; n= number of patients; CI= confidence interval; P= significance; RDT= rapid diagnosis test; ICT= Immuno-chromatographic test.

## CONCLUSION

Early diagnosis is not only important for better clinical management of dengue patients but prevents unnecessary usage of antibiotics, thus providing epidemiology data for guidance of health policy decisions especially once dengue vaccines and antivirals become a reality. Understanding the clinical conditions of dengue patients and the pattern of immune response is essential for appropriate usage of current dengue diagnostics. Assays that are developed need to be sensitive, detectable as early as possible after onset of fever and have minimal cross-reactivity with other circulating flaviviruses. In addition it is essential to keep inter-assay and intra-assay variability to a minimum, with costs, simplicity and rapidity being other factors that must be considered when developing a diagnostic test. Newer technologies using biosensors that can be qualitative, quantitative, rapid, sensitive and specific are in development and also may have desirable traits such as being portable, can be automated and can be easily disposed. However their sensitivity and specificity have yet to be fully validated and currently are not fully compliant with the basic requirements of a rapid diagnostic test as gaps exist with regards to their field applicability, availability and affordability as a point of care test.[57-60] Generally biosensor kits developed have not met the validity and requirements of a rapid test for dengue. Future dengue assays are hoped to go beyond and also include surrogate markers for disease severity.

**Acknowledgements**
The author thanks Ravi Chandra Vemuri and Rohit Gundamaraju for the preparation of the tables and figure and for editorial assistance.

## REFERENCES

1. World Health Organization. Dengue:Guidelines for Diagnosis, Treatment, Prevention and Control 2009.WHO/HTM/NTD/DEN/2009.1.
2. Beatty ME, Stone A, Fitzsimons DW, et al. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas Dengue Prevention Boards. PLoS Negl Trop Dis 2010; 4: e890.
3. World Health Organization: Western Pacific Regional Office 2015. Available at: http://reliefweb.int/sites/reliefweb.int/files/resources/dengue_biweekly_20150408.pdf
4. Ministry of Health, Malaysia. iDengue database (2015). Available: http://idengue.remotesensing.gov.my/idengue/index.php
5. Gutie´rrez G, Gresh L, Pe´rez MA´ , Elizondo D, Avile´s W, et al. Evaluation of the Diagnostic Utility of the Traditional and Revised WHO Dengue Case Definitions. PLoS Negl Trop Dis 2013; 7: e2385.
6. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998; 11, 480–496
7. Innis BL. Dengue and dengue hemorrhagic fever. In: Porterfield, JS eds. Exotic Viral Infections. London: Chapman & Hall, 1995: 103–146.
8. Sekaran SD, Artsob H. Molecular diagnostics for the detection of human flavivirus infections. Expert Opinion on Medical Diagnostics 2007; 1: 521-30.
9. Dussart P, Baril L, Petit L, et al. Clinical and virological study of dengue cases and the members of their households: the multinational DENFRAME Project. PLoS Negl Trop Dis 2012; 6:e1482.

10. Mathew A, Rothman AL. Understanding the contribution of cellular immunity to dengue disease pathogenesis. Immunol Rev 2008; 225:300-13.
11. Guzman MG, Halstead SB, Artsob H, et al. Dengue: a continuing global threat. Nat Rev Microbiol 2010; 8:S7-16.
12. Peeling RW, Artsob H, Pelegrino JL, et al. Evaluation of diagnostic tests: dengue. Nat Rev Microbiol 2010; 8:S30-8.
13. Halstead SB. Dengue. Lancet 2007; 370:1644-52.
14. Kong YY, Thay CH, Tin TC, et al. Rapid detection, serotyping and quantitation of dengue viruses by TaqMan real-time one-step RT-PCR. J Virol Methods 2006; 138:123-30.
15. Yong YK, Thayan R, Chong HT, et al. Rapid detection and serotyping of dengue virus by multiplex RT-PCR and real-time SYBR green RT-PCR. Singapore Med J 2007; 48:662-8.
16. Jarman RG, Nisalak A, Anderson KB, et al. Factors influencing dengue virus isolation by C6/36 cell culture and mosquito inoculation of nested PCR-positive clinical samples. Am J Trop Med Hyg 2011; 84:218-23.
17. Gibbons RV, Vaughn DW. Dengue: an escalating problem. BMJ 2002 324:1563–1566.
18. Leparc-Goffart I, Baragatti M, Temmam S, et al. Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses. J Clin Virol 2009; 45:61-6.
19. Hue KD, Tuan TV, Thi HT, et al. 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 2011; 177:168-73.
20. Usawattanakul JA, Tapchaisri P, Siripanichgon K, Buchachart K, Hong-ngarm A, Thongtaradol P, Endy TP. Detection of Dengue Viral RNA in Patients' Sera by Nucleic Acid Sequence-Based Amplification (NASBA) and Polymerase Chain Reaction(PCR). Dengue Bull 2002; 26: 131-9.
21. Wu SJ, Lee EM, Putvatana R, et al. Detection of dengue viral RNA using a nucleic acid sequence-based amplification assay. J Clin Microbiol 2001; 39:2794-8.
22. Compton J. Nucleic acid sequence-based amplification. Nature 1991; 350:91-2.
23. Parida M, Horioke K, Ishida H, et al. Rapid Detection and Differentiation of Dengue Virus Serotypes by a Real-Time Reverse Transcription-Loop-Mediated Isothermal Amplification Assay. J Clin Microbiol 2005; 43:2895-903.
24. Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother 2009; 15:62-9.
25. Munoz-Jordan JL, Collins CS, Vergne E, et al. Highly sensitive detection of dengue virus nucleic acid in samples from clinically ill patients. J Clin Microbiol 2009; 47:927-31.
26. Libraty DH, Young PR, Pickering D, et al. 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 2002; 186:1165-8.
27. Avirutnan P, Punyadee N, Noisakran S, et al. Vascular Leakage in Severe Dengue Virus Infections: A Potential Role for the Nonstructural Viral Protein NS1 and Complement. J Infect Dis 2006; 193:1078-88.
28. Pal S, Dauner AL, Mitra I, Forshey BM, Garcia P, Morrison AC, Wu SJL. Evaluation of Dengue NS1 Antigen Rapid Tests and ELISA Kits Using Clinical Samples. PloS one 2014; 9(11):e113411.
29. Shenoy B, Menon A, Biradar S. (2014). Diagnostic utility of dengue NS1 antigen. Ped Infect Dis 2014; 6:110-3.
30. Naz A, Zahid D, Murky SN, Nadeem M, Sil BK, Shamsi TS. Evaluation of efficacy of various immunochromatographic rapid tests for dengue diagnosis. Pak J Med Sci 2014; 30:166-71.
31. Bessoff K, Delorey M, Sun W, Hunsperger 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 2008; 15:1513–18.
32. Dussart P, Petit L, Labeau B, Bremand L, Leduc A, et al. Evaluation of two new commercial tests for the diagnosis of acute dengue virus infection using NS1 antigen detection in human serum. PLoS Negl Trop Dis 2008; 2:e280.
33. Blacksell SD, Jarman RG, Gibbons RV, Tanganuchitcharnchai A, Mammen MP, et al. Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection. Clin Vaccine Immunol 2012; 19:804-10.
34. Aryati A, Trimarsanto H, Yohan B, Wardhani P, Fahri S, Sasmono RT (2013) Performance of commercial dengue NS1 ELISA and molecular analysis of NS1 gene of dengue viruses obtained during surveillance in Indonesia. BMC Infect Dis 2013; 13: 611.
35. Pal S, Dauner AL, Valks A, Forshey BM, Long KC, Thaisomboonsuk B, Wu SJL. Multicountry Prospective Clinical Evaluation of Two Enzyme-Linked Immunosorbent Assays and Two Rapid Diagnostic Tests for Diagnosing Dengue Fever. J Clin Microbiol 2014; 53:1092-1102.
36. Pan-ngum W, Blacksell, SD, Lubell Y, Pukrittayakamee S, Bailey, M. S, de Silva, et al. Estimating the true accuracy of diagnostic tests for dengue infection using bayesian latent class models. PloS one 2013; 8:e50765.
37. Ferraz FO, Bomfim MRQ, Totola AH, Ávila TV, Cisalpino D, Pessanha JEM, et al. Evaluation of laboratory tests for dengue diagnosis in clinical specimens from consecutive patients with suspected dengue in Belo Horizonte, Brazil. J Clin Virol 2013; 58:41-6.
38. Sánchez-Vargas, LA, Sánchez-Marce EE, Vivanco-Cid, H. Evaluation of the SD BIOLINE

dengue duo rapid test in the course of acute and convalescent dengue infections in a Mexican endemic region. Diagn Microbiol Infect Dis 2014; 78:368-72.
39. Guzman MG, Jaenisch T, Gaczkowski R, Hang VTT, Sekaran SD, Kroeger A, et al. Multi-country evaluation of the sensitivity and specificity of two commercially-available NS1 ELISA assays for dengue diagnosis. PLoS neglected tropical diseases 2010; 4:e811.
40. Wang SM, Sekaran SD. (2010). Early diagnosis of Dengue infection using a commercial Dengue Duo rapid test kit for the detection of NS1, IGM, and IGG. Am J Trop Med Hyg 2010; 83:690-95.
41. Sekaran SD, Ew CL, Kanthesh BM, Appanna R, Subramaniam G. Evaluation of dengue NS1 capture ELISA assay for the rapid detection of dengue. J Infect Developing Countries 2007; 1:182–188.
42. Wang SM, Sekaran SD. 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 2010; 48:2793-97.
43. Anders KL, Nguyet NM, Quyen NTH, Van Ngoc T, Van Tram T, Gan TT, Simmons CP. An evaluation of dried blood spots and oral swabs as alternative specimens for the diagnosis of dengue and screening for past dengue virus exposure. Am J Trop Med Hyg 2012; 87:165-70.
44. Libraty DH, Young PR, Pickering D, et al. 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 2002; 186:1165-8.
45. Avirutnan P, Punyadee N, Noisakran S, et al. Vascular Leakage in Severe Dengue Virus Infections: A Potential Role for the Nonstructural Viral Protein NS1 and Complement. J Infect Dis 2006; 193:1078-88.
46. Xu H, Di B, Pan YX, et al. Serotype 1-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: Implications for early diagnosis and serotyping of dengue virus infections. J Clin Microbiol 2006; 44:2872-8.
47. Pal S, Dauner AL, Valks A, Forshey BM, Long KC, Thaisomboonsuk B, Wu SJL. Multicountry Prospective Clinical Evaluation of Two Enzyme-Linked Immunosorbent Assays and Two Rapid Diagnostic Tests for Diagnosing Dengue Fever. J Clin Microbiol 2014; 53:1092-1102.
48. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria, DA, et al. (2009). Evaluation of commercially available anti-dengue virus immunoglobulin M tests. Emerg Infect Dis 2009; 15:436-40.
49. Chuansumrit A, Chaiyaratana W, Tangnararatchakit K, et al. Dengue nonstructural protein 1 antigen in the urine as a rapid and convenient diagnostic test during the febrile stage in patients with dengue infection. Diagn Microbiol Infect Dis 2011; 71:467-9.
50. Vaughn DW, Green S, Kalayanarooj S, et al. Dengue Viremia Titer, Antibody Response Pattern, and Virus Serotype Correlate with Disease Severity. J Infect Dis 2000; 181:2-9.
51. Lam SK, Devi S, Pang T. Detection of specific IgM in dengue infections. Southeast Asian J Trop Med Pub Health 1987; 18:532-8.
52. Chong CF, Ngoh BL, Tan HC, et al. A shortened dengue IgM capture ELISA using simultaneous incubation of antigen and peroxidase-labeled monoclonal antibody. Clin Diagn Virol 1994; 1:335-41.
53. Nunes MR, Nunes Neto JP, Casseb SM, et al. Evaluation of an immunoglobulin M-specific capture enzyme-linked immunosorbent assay for rapid diagnosis of dengue infection. J Virol Methods 2011; 171:13-20.
54. Talarmin A, Labeau B, Lelarge J, et al. Immunoglobulin A-specific capture enzyme-linked immunosorbent assay for diagnosis of dengue fever. J Clin Microbiol 1998; 36:1189-92.
55. Ahmed F, Mursalin H, Alam MT, et al. Evaluation of ASSURE(R) Dengue IgA Rapid Test using dengue-positive and dengue-negative samples. Diagn Microbiol Infect Dis 2010; 68:339-44.
56. de la Cruz Hernández SI, Mateos SG, Aguilar H F, Martinez IL, Aranda CA, et al. Evaluation of a novel commercial rapid test for dengue diagnosis based on specific IgA detection. Diagn Microbiol Infect Dis 2012; 72:150-5.
57. Wu TZ, Su CC, Chen LK, et al. Piezoelectric immunochip for the detection of dengue fever in viremia phase. Biosens Bioelectron 2005; 21:689-95.
58. Baeumner AJ, Schlesinger NA, Slutzki NS, et al. Biosensor for Dengue Virus Detection: Sensitive, Rapid, and Serotype Specific. Anal Chem 2002; 74:1442-8.
59. Dodeigne C, Thunus L, Lejeune R. Chemiluminescence as diagnostic tool. A review. Talanta 2000; 51:415-39.
60. Atias D, Liebes Y, Chalifa-Caspi V, et al. Chemiluminescent optical fiber immunosensor for the detection of IgM antibody to dengue virus in humans. Sens Actuators B Chem 2009; 140:206-15.