Review of Current Laboratory Diagnostics for Dengue Fever

Appavu K. Sundaram*, Cheng-Rei R. Lee, and Shuenn-Jue L. Wu
Department of Viral and Rickettsial Diseases, Naval Medical Research Center, USA

Abstract

Early and accurate diagnosis of dengue fever (DF), the leading arthropod-borne viral disease, is critical for patient triage, better patient management, surveillance, pathogenicity and vaccine research studies. DF presents as an undifferentiated fever initially and exhibits DF-specific symptoms as the disease progresses. Laboratory diagnostic assays are useful during the early acute phase of infection when viremia is observed as well as during the early convalescent phase when IgM and IgG antibodies are present. If the patients are not diagnosed early and given intensive supportive care, most cell degranulation in response to dengue virus (DENV) infection and antibody dependent enhancement effects during the secondary infections could lead to severe form of the disease, marked by internal bleeding and vascular leakage, and result in mortality. This aspect emphasizes the importance of an accurate DF diagnosis at early stage of the infection. Currently, most laboratory dengue diagnosis is achieved by DENV isolation, DENV RNA detection, ELISA for anti-DENV IgM and IgG antibodies and DENV non-structural protein 1 (NS1) as well as lateral flow immunochromatographic tests (ICTs) for anti-DENV IgG and IgM antibodies and NS1 antigen. Novel technologies and methods that show great potential for improved DF diagnostics in the future are also briefly discussed.

ABBREVIATIONS

DENV: Dengue Virus; DF: Dengue Fever; ELISA: Enzyme-Linked Immunosorbent Assay; ICT: Immunochromatographic Test; IFA: Immunofluorescence Assay; LAMP: Loop-Mediated Isothermal Amplification; LFA: Lateral Flow Assay; NA: Nucleic Acid; NS1: Non-Structural Protein 1; POC: Point-Of-Care; POS: Post Onset of Symptoms; PRNT: Plaque Reduction Neutralization Test; RDT: Rapid Diagnostic Test; RT-PCR: Reverse Transcription Polymerase Chain Reaction; rRT-PCR: Real-Time Reverse Transcription Polymerase Chain Reaction; SD: Severe Dengue

INTRODUCTION

Dengue fever (DF) is the leading arthropod-borne viral disease caused by dengue virus (DENV), a single stranded, enveloped RNA virus. DENV is a group of viruses comprised of four related but genetically distinct serotypes (DENV 1-4), belonging to the genus Flavivirus in the Flaviviridae family [1-3]. Infection by one DENV serotype confers long-term immunity to that serotype but not to the other three serotypes. However, antibodies generated against each of the four DENV serotypes are cross-reactive and only confer partial and short-term cross-protective immunity [4]. DENV infection has expanded globally with sustained outbreaks in Southeast Asia, the Americas, the Western Pacific, Africa and the Eastern Mediterranean regions and is one of the fastest growing global health concerns [5]. DF is endemic to more than 100 countries, especially in tropical and subtropical regions [5,6]. Initial clinical presentation of DF is similar to most acute febrile illnesses including malaria, chikungunya, Zika, leptospirosis, and influenza [7-9]. Most DENV infections result in asymptomatic or subclinical infections and the majority of the patients recover after a self-limiting DF illness [10]. However, 5 – 10% of patients progress to severe dengue (SD), previously known as dengue hemorrhagic fever [11,12]. SD is characterized by plasma leakage, internal bleeding, respiratory distress and organ impairment. Currently, there is no dengue-specific therapeutics and DF prevention is limited mostly to vector control, in spite of the first dengue vaccine (Dengvaxia by Sanofi Pasteur) being recommended for use only in endemic areas [5,13]. Therefore, early diagnosis of DF is important for monitoring the patients and alerting the health centers for providing appropriate hospitalization and supportive therapy for patients who may progress to exhibit the severe form of the disease [5]. Accurate and efficient diagnosis of DF is therefore important not only for clinical care, surveillance support, pathogenesis studies, and vaccine research but also for case confirmation [14]. In this review, we describe the current status of dengue diagnostics as well as novel technologies and methods that show great potential for improved diagnostics in the future.

CURRENT LABORATORY DIAGNOSTICS

In the acute phase of dengue illness, 0 – 7 days post onset of symptoms (POS), dengue patients present with sudden onset of fever, accompanied by nausea, aches, pains and rashes. However, these symptoms are not unique to DF and are also exhibited by some other febrile diseases. Therefore, a definitive diagnosis of DF warrants a laboratory confirmation which can be performed best during the acute phase of the infection. Laboratory diagnosis
for DENV infections is mainly based on three methods: virus isolation, nucleic acid (NA) detection, and immunoassays to detect either DENV-specific antigens or anti-DENV antibodies. Dengue viral RNA and soluble DENV antigens are detectable in patient's blood during the early acute phase of infection (0 – 5 days POS), when viremia is high. Therefore, both virus isolation and RNA detection are only useful during the acute viremia phase of DENV infection. Immunoassays, on the other hand, could cover detection during all phases of DENV infections, depending on the assay target component(s) [15-18].

Virus isolation

Traditional laboratory DF diagnosis is based on virus isolation followed by immunofluorescence assay (IFA) using serotype-specific anti-DENV monoclonal antibodies (MAbs) to identify the serotype of the isolated virus [4]. Dengue viremia has been observed 2 days before onset of fever and up to 4 and 5 days POS in primary and secondary infections, respectively [19,20]. Therefore, within 0 – 4 days POS, DENV can be isolated from blood, serum and plasma. In general, virus isolation rates range from 71.5 – 84 % depending on the DENV serotype, and can be as high as 91 % if the specimen is collected early during the infection course [19,21]. Furthermore, virus isolation has been found to be more successful in primary infections than in the secondary infections, probably due to the neutralizing effects of pre-existing anti-DENV antibodies [19]. Virus isolation in the laboratory is carried out by inoculating mosquito cell lines C6/36 or mammalian cell lines, such as Vero, LLC-MK2 and BHK-21, with clinical specimens from DF suspected patients [19]. Diagnosis of DF by virus isolation is highly specific, as the isolated virus is confirmed and serotyped by IFA using serotype-specific anti-DENV MAbs. A major disadvantage of this method is that virus isolation can take several days to weeks for completion. Other disadvantages of viral isolation are that it requires highly trained personnel, and sample stability and integrity are critical for successful virus isolation [19].

Viral RNA detection

Successful extraction and isolation of DENV RNA from the blood, serum or plasma of the patient followed by NA amplification and identification by reverse transcription polymerase chain reaction (RT-PCR) is also considered as a gold standard for DF diagnostics much like the viral isolation method. Both RT-PCR and real-time RT-PCR (rRT-PCR) can be used for sensitive and specific detection of DENV RNA in the clinical samples. Therefore, RT-PCR based methods are useful in diagnosing DF only during the early acute phase of infection, just like the virus isolation, but can be completed in as little as 2 hours. A number of RT-PCR assays utilizing specific primer sets and probes for different regions of the DENV genome have been developed for detection and/or identification of DENV serotypes with high sensitivity and specificity. Lanciotti et al, developed a rapid hemi-nested PCR assay for both universal detection of DENV and the serotype-specific detection of DENV 1-4 [22]. In this assay, universal consensus primers to all of the 4 DENV serotypes were used to generate a 511-bp product in the first round by RT-PCR. This RT-PCR product was then serotyped in the second round using serotype-specific primers. Sudiro et al., designed a pair of universal primers covering the 3’-non-coding region of DENV 1-4 and developed an rRT-PCR assay that amplified DENV-specific RNAs [23]. Later McAvin et al., used both universal and serotype-specific primers and probes and developed an rRT-PCR assay for serotype-specific detection and identification of DENV in mosquito vectors and human sera for epidemiological surveillance [24,25]. Although several such RT-PCR based methods have been developed to detect and identify DENV RNA in patient blood, they were mostly used as research tool and not as dengue diagnostic tools [26,27].

Santiago et al., from the US Centers for Disease Control and Prevention (CDC), designed and developed the only dengue real-time RT-PCR assay (CDC DENV 1-4 rRT-PCR)that has been cleared by the US Food and Drug Administration (FDA) as an in vitro diagnostic device for the diagnosis of DF (for serotype-specific detection of DENV in human serum or plasma) [28]. The CDC DENV 1-4 rRT-PCR assay is intended for use on an Applied Biosystems (ABI) 7500 Fast Dx real-time PCR instrument. A positive control virus mixture and a non-infectious human specimen control (RNA extraction control) are included in the kit. The CDC rRT-PCR can be run either as a singleplex or a multiplex assay. During the primer design for the CDC rRT-PCR assay, enough degeneracy was introduced in a given nucleotide position to accommodate various strains of each DENV serotype, in order to minimize the number of false negatives due to strain variation. The CDC rRT-PCR assay amplifies the NS5 region of DENV 1, the E protein region of DENV 2, and different regions of the prM region of DENV 3 and DENV 4. Although the CDC DENV 1-4 rRT-PCR is the only FDA-cleared DENV RNA detection assay, it has been shown to be less sensitive, with a limit of detection (LOD) of 10^4 pfu/mL, than most of the laboratory-based RT-PCR assays. Waggoner et al., compared their laboratory-developed DENV multiplex rRT-PCR assay with CDC DENV 1-4 rRT-PCR assay using clinical samples and showed that their assay exhibited a higher clinical sensitivity than the CDC assay [29–31].

Altona Diagnostics (Hamburg, Germany) markets a pan-serotype Real Star dengue RT-PCR kit that is CE marked [32]. Focus Diagnostics Simplex Dengue rRT-PCR is intended for use on the 3M Integrated Cycler instrument for the detection and serotyping of DENV 1-4 in human serum [32]. Focus Diagnostics’ assay amplifies four serotype-specific regions of the DENV (NS5 genes in DENV 1 and DENV 3, NS3 gene in DENV 2 and capsid gene in DENV 4) and discriminates DENV 1 and DENV 4 in one reaction well and DENV 2 and 3 in another reaction well. A positive control reagent is also provided in this kit. DENV pan-serotype rRT-PCR kitLiferiver (Shanghai Z Bio-Tech Co., Shanghai, China) has been developed for use with LightCycler 1.0/2.0/480 instruments [32]. This commercial kit also includes a positive control reagent at a concentration of 10^8 copies/mL. LGeno-Sen’s DENV 1-4 rRT-PCR kit (Genome Diagnostics Pvt, New Delhi, India) is developed for use in the Rotor Gene Thermocycler and can be adapted to an ABI7500 instrument [32]. This assay does not differentiate between serotypes. Internal positive controls are provided in this kit at concentrations 10^6 – 10^8 copies/mL. Evaluation of all four commercial DENV rRT-PCR kits using a panel of 162 serum samples showed that the Simplex Dengue rRT-PCR exhibited the best performance whereas the Liferiver RT-PCR kit showed poor sensitivity [32]. Clinical sensitivity of the Simplexa kit was found
to be 93.2%, whereas the sensitivity of Geno-Sen’s and the Real Star kits were found to be 85.2% and 83.3%, respectively. Since the Lifefirst kit showed a poor sensitivity in the initial panel of 40 samples (only 28 DENV positive), it was not evaluated further. Although all four rRT-PCR kits are marketed for DF diagnostics they are not to be used in the US for diagnostic purposes since they do not have FDA clearance.

Conventional RT-PCR based methods require a trained operator and an expensive thermal cycler. In addition, a laborious NA extraction and purification step is also required to avoid PCR inhibition by integral components of the sample matrix. Due to these limitations, RT-PCR based methods are not currently used for point-of-care (POC) DF diagnostics and are best suited for diagnostic reference laboratories. Several isothermal amplification methods, including nucleic acid sequence based amplification (NASBA) and reverse transcription loop-mediated isothermal amplification (RT-LAMP), have been developed in order to address these limitations and to simplify the diagnostic device complexity so that they can be fielded as POC diagnostic devices [33-36]. These isothermal NA detection methods are described in detail in the future perspectives of DF diagnostics section of this review.

Immunoassays

Immonoassay-based detection of DENV infection can be divided into two categories: DENV antigen detection and anti-DENV antibody detection. DENV antigen detection assay detects DF during the acute phase while the anti-DENV antibody detection assays may not be positive in the early acute phase of the disease [37]. The sensitivity and specificity of immunoassay-based diagnosis of DF varies depending on the disease state (days POS), primary vs secondary infection, the target analyte (s) in the test, and the DENV serotype [38]. DENV has three structural proteins: the capsid protein (C), the envelope protein (E), and the membrane protein (M); and seven non-structural (NS) proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 [3]. Among the DENV antigens, NS1 is highly conserved, is released during viremia in soluble form, can be found in concentrations as high as 50 µg/mL in human serum, and can be detected in both primary and secondary DENV infections [39,40]. It is not surprising that NS1 is currently regarded as the most prominent biomarker for diagnosis of DF during the acute phase [41].

For anti-DENV antibody detection, current DF diagnostics mostly focus on anti-DENV IgM and IgG. Anti-DENV IgM is detectable 4 – 5 days POS regardless of primary or secondary DENV infection, albeit at lower levels in secondary infections than primary infections. Anti-DENV IgM appears before anti-DENV IgG for primary cases whereas IgG precedes IgM production in secondary cases. Anti-DENV IgG peaks at a higher level and persists longer than IgM for both primary and secondary infections and peaks faster and reaches much higher levels in secondary cases [42]. Anti-DENV antibodies cross-react to some extent with other flavivirus such as Japanese encephalitis virus, St. Louis encephalitis virus, West Nile virus, and yellow fever virus [43]. Patient sera with PCR-confirmed DENV infection were also found to be cross-reactive with Zika virus (ZIKV) in antibody binding assay by enzyme-linked immunosorbent assay (ELISA) [44].

Plaque reduction neutralization test (PRNT), is a highly specific and sensitive serological test for determining the immunoprotection against DENV [45]. PRNT is often used to diagnose a current dengue infection as it demonstrates seroconversion in paired sera samples from suspected patients, indicated by a fourfold or greater increase in antibody titers. Although, PRNT is commonly used in the laboratories during the development and evaluation of vaccines, it is not routinely used in DF diagnostics since it is expensive and time consuming [45]. More widely used immunoassays for current DF diagnostics include capture ELISA and rapid diagnostic tests (RDTs) for NS1 and IgM/IgG [46]. The popularity of ELISA is due to its simplicity, reproducibility, and ability for automation [37]. RDTs are mostly lateral flow assays (LFA), also referred to as immunochromatographic tests (ICTs) [47,48]. The detection format for RDTs can vary and the advantages, as a POC diagnostic device, are great when compared to ELISA: rapidity, one-step analysis, low cost, simple/no instrumentation, and user and field friendly formats to name a few [49]. RDTs can provide results in as little as 15 min [38]. There are plenty of ELISA and RDT products available on the market with a wide range of performance specifications. For examples, six commercial dengue-specific IgM ELISAs require an assay time of 90-225 min with sensitivity and specificity of the assay ranging from 61.5-98.6% and 79.9-97.3%, respectively, whereas four commercial dengue-specific IgM RDTs have a range of 15-90 min for assay time, with 20.5-97.7% for sensitivity, and 62.2-90.6% for specificity. When the assay target was changed to NS1, assay time was in the range of 111-160 min/15-30 min with sensitivity ranged in 72.3-95.9%/70.6-86%, and specificity ranged in 95.5-100%/73.4-100% for ELISAs/RDTs, respectively [50]. Blacksell et al., evaluated 6 commercial RDTs using 259 acute fever (dengue and non-dengue) patient samples and found that NS1 RDTs had sensitivity and specificity at 49-59% and 93-99%, respectively, while anti-DENV IgM RDTs had 71-80% sensitivity and 46-90% specificity [47]. A meta-analysis of 18 studies was performed on NS1-based ELISA and ICT kits and summarized that sensitivity and specificity were 67% and 99% for ELISA kits and 71% and 99% for ICT kits [51]. Although, ELISAs are more sensitive, the lack of portability prevents their use in POC/field settings and are mostly recommended for use in laboratory settings where trained personnel and necessary equipment are available [50,52]. Antibody detection-based immunoassays targeting anti-DENV IgA have also been explored for covering early acute phase of DF. Naz et al., evaluated ASSURE Dengue IgARDT kit, and found that this kit exhibited a sensitivity of 85.21% and specificity of 80.85%, and could detect both primary and secondary dengue infections comparatively better than commercially available NS1 and IgG/IgM RDTs and ELISAs [48]. Patela et al. evaluated Dengue IgA Capture ELISA by BIO-RAD was evaluated by De Decker et al. and found to show an overall 93% sensitivity and 88% specificity [53]. However, the overall sensitivity of anti-dengue IgA detection was the lowest among the 4 analytes (NS1, IgM, IgA, and IgA) tested in 6 commercial RDTs using saliva samples [54].

Dengue virus NS1 RDTs are advantageous over DENV RNA tests and IgG/IgM ELISA detections due to low cost, ease of testing, simplicity of devices, user friendliness, and suitability for resource-limited settings [52,55]. However, sensitivity of NS1 antigen detection can vary greatly between different serotypes,
averaging a 50% for DENV4 as compared to a 93% for DENV1 from 4 RDTs tested [52]. It can also be compromised in secondary dengue infections as pre-existing anti-NS1 antibodies may bind to the circulating NS1 and form antigen-antibody complexes, thereby reducing the freely available NS1 antigen concentration in the samples [56]. Therefore, it is apparent that there is no one single test that can achieve high enough sensitivity for DF diagnostics and the best positivity of DF can be captured by combining anti-DENV antibody detection with DENV RNA or NS1 detection [55,57]. A retrospective study in Asia showed sensitivities of 62% and 72.5% for NS1 and IgG/IgM RDTs, respectively when used separately. However, in the same study, when these NS1 and IgG/IgM RDTs were used together, the sensitivity improved to 93%, without compromising the specificity [46]. A Sri Lanka cohort study also revealed the best sensitivity and specificity combination (93% and 89%) by Standard Diagnostics Dengue Duo (NS1 and IgM/IgG) test kit [47]. The findings by Pal et al’s multicenter evaluation of two ELISAs and two RDTs showed that by combining NS1 and IgM/IgG detection in one diagnostic device clinical performance (sensitivity) of the tests can be significantly improved [38]. Such combined immunoassays to detect both the presence of DENV antigen and anti-DENV antibodies not only provide the much needed clinical performance boost, but also expand the detection window from as early as 1 day POS (early acute phase) to late convalescent phase [46]. Some of the currently available immunoassay-based POC dengue diagnostic tests are manufactured by Alere, Zephyr Biomedicals, InBios, Core Diagnostics, Bio-Rad, and Focus Diagnostics. However, at present, there is no FDA-cleared dengue POC test.

**FUTURE PERSPECTIVES OF DENGUE DIAGNOSTICS**

**Isothermal amplification**

Molecular techniques such as RT-PCR and nested PCR are useful for confirmatory diagnosis of DENV infections including serotype-specific identifications performed in the reference laboratories. However, the need for expensive and complex thermal cyclers in addition to the cold storage requirement for the PCR reagents has significantly hampered the use of these methods in resource-limited countries and POC settings. NASBA is an isothermal RNA-specific amplification assay that does not need thermal cyclers. In this method, initially a single-stranded RNA molecule is copied into a double stranded DNA molecule by reverse transcription [33,34]. This amplicon (double stranded DNA) is then detected by electrochemiluminescence or fluorescent-labelled molecular probes. LAMP is another efficient DNA amplification method under isothermal conditions, that is simple and easy to use [35]. Briefly, the inherent strand displacement activity and the polymerase activity of the Bst polymerase is utilized in the LAMP technology for both primer annealing and DNA amplification at the same temperature, by utilizing primers that initiate a specific stem loop structure when binding to a target structure [36]. When combined with reverse transcription, isothermal amplification methods have a great potential to be used as a simple dengue diagnostic device especially at resource-limited settings. Several research groups have been working on developing RT-LAMP based diagnostics for DF. Although a variety of detection methods such as by UV light, or by using DNA intercalating dye SYBR Green, are available to visualize the amplified DNA, a reliable detection method that can differentiate the background signal from the real signal arising from target-specific amplified products is yet to be identified [36]. Despite this major limiting factor, RT-LAMP based methods have seen much anticipated advances within the last decade and several companies have invested in developing RT-LAMP based diagnostic devices for POC use.

**Multiplexed assays**

Recent emergence of ZIKV in parts of South America warrants further research and development of multiplexed assays to not only detect DF but also to differentiate between other related febrile illnesses such as malaria, chikungunya, and Zika in co-circulating endemic regions [9,44]. The cross-reactivity of dengue diagnostic devices with Zika and chikungunya is also a major concern [44]. The majority of dengue POC diagnostic devices developed before the emergence of ZIKV may have to be re-evaluated to ensure that there is no misdiagnosis. A multiplexed LFA was designed using two-color detection probes to enable multiplexed detection of IgG/IgM for DENV and chikungunya virus (CHIKV) on a single assay strip, by researchers from Cornell University [58]. Based on the color (red, blue or mix of red and blue) and the location of the test dots observed, the test can indicate the presence of anti-CHIKV and anti-DENV IgG/IgM. Similar multiplexed assays for differential diagnosis of CHIKV, DENV and ZIKV infection are being developed and evaluated by bioLytical Laboratories (Richmond, Canada).

Moving on to the new frontier for DF diagnostics, a non-invasive, stacking flow immunoassay technology has been developed to detect DENV-specific IgG in saliva, with reduced background and reliable quantification to aid in differentiating between primary and secondary infections [59]. A portable microfluidic device has been designed, based on this technology, at Singapore’s A*STAR research agency in 2015 (www.madgadget.com) Professor George Whitesides’ group at Harvard University has developed a similar, paper-based POC diagnostic device that can be adapted to develop a dengue POC diagnostic device [60-62]. Whitesides group is currently applying this technology for developing a cost-effective dengue POC diagnostic test for use in resource limited countries (personal communication). In order to increase the sensitivity of paper-based immunoassay, signal amplification methods have been studied extensively [63]. Researchers from Massachusetts Institute of technology used silver nanoparticles for their paper-based tests intending to multiplex their prototype by showing bands, in different color, corresponding to different diseases including Ebola, DF, and yellow fever. An automated serum NS1 quantification assay, using NS1 coated magnetic nanoparticles (MNPs), takes just 8 minutes for completing the test, requires 6 μL of serum sample and showed an LOD of 25 ng/mL with an upper detection limit of 20,000 ng/mL [64]. Vertical flow rapid diagnostic tests have been developed for diagnosis of various infectious diseases such as leptospirosis (using human anti-leptospirosis IgM) with 89.8% sensitivity and 93.7% specificity [65]. By changing from lateral to vertical flow, the test time can be reduced significantly, (about 15-30 minutes for lateral flow vs 1-2 minutes for vertical flow). The potential application of such technologies for POC detection of
flavivirus infections including DF is being evaluated by MedMira (Halifax, Canada) and biolytical Laboratories.

Rapid tests to predict progression to SD

Although the majority of DF patients recover spontaneously, about 5 – 10% of dengue cases progress to SD, especially in secondary infections. SD has become the leading cause of hospitalization and death in many Asian and Latin American countries. Fatalities associated with SD can be reduced if the patients who are likely to progress to SD can be identified early, monitored for warning symptoms and given intensive supportive therapy in a timely manner [5]. Therefore, the outcome of clinical management for SD patients relies heavily on the ability to quickly diagnose and to start proper treatment early. Although there is no reliable prognostic test for SD at present, a few biomarkers have been identified as potential candidates for SD prediction [11,66-74]. Of those biomarkers, Interleukin-10 (IL-10) and chymase have been further evaluated by ELISA using acute phase clinical samples [11,75]. Development of RDTs, as companion diagnostics, using such biomarkers to predict progression to SD will be of great help in reducing the SD associated mortality rate by monitoring those patients and giving them the required supportive therapy.

CONCLUSION

Dengue virus infection is a growing global health concern. 75% of DENV infections are asymptomatic while most of the clinically symptomatic patients exhibit a self-limiting DF illness. However, some DF patients proceed to a severe form of the disease and if not monitored and given an intensive supportive therapy it may result in a high fatality rate. Therefore, early and accurate diagnosis of DF is essential for patient triage. Virus isolation is the gold standard dengue diagnostic method, but is significantly hampered because the method is laborious and very time consuming. Although DENV detection by RT-PCR can be completed in less than 2 hours, it requires a highly skilled operator and expensive instruments, in addition to requiring an exhaustive sample preparation method to minimize contamination in the laboratory environment. Immunoassays based NS1 and/or anti-DENV IgM/IgG detection methods have been developed in simple lateral flow formats for rapid and accurate detection of DENV infections at different stages of illnesses. Although many POC dengue diagnostics are commercially available, none of them are FDA-cleared for use in the US. The ideal POC rapid test criteria for diagnosing infectious diseases in the developing world formulated by Peeling, remains true today: Affordable, Sensitive, Specific, User-friendly, Robust and Rapid, Equipment-free or minimal, Deliverable (ASSURED) to those who need them [17]. Reduced background signal, high sensitivity/specificity, and the ability to multiplex remain the major challenges in developing effective dengue POC tests. A multiplexed dengue diagnostic POC device that combines both NS1 and anti-DENV IgM/IgG detection will be of great significance, since that test will cover a broad window of DF detection (early acute phase to late convalescent phase) ranging from 0 - 15 days POS. Development of RDTs to predict SD progression as companion diagnostics will help healthcare facilities and hospitals in resource-limited countries greatly by enabling specific monitoring of patients who are likely to progress to the severe form of the disease.

DISCLAIMER

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