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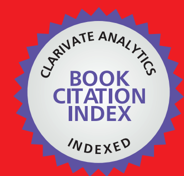

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Chapter 4

Diagnosis of Dengue: Strengths and Limitations of Current Techniques and Prospects for Future Improvements

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Additional information is available at the end of the chapter

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Abstract

Dengue is an increasingly common mosquito-borne, flaviviral disease of significant public health concern. The disease is endemic throughout tropical and subtropical regions, placing almost half of the world's population at risk, and each year approaching 100 million people in around 130 countries are infected. There is acknowledged to be four antigenically distinct serotypes of the virus, and arguably a fifth, each of which elicits a full spectrum of clinical disease. This ranges from asymptomatic self-limiting infection to life-threatening severe manifestations characterized by plasma leakage, bleeding, and/or organ failure. Recovery from primary infection by one serotype provides lifelong immunity against reinfection by that particular serotype, whereas with subsequent infections by other serotypes, the risk of developing severe dengue is increased. Rising mortality and morbidity rates caused by infection in recent years are attributable partly to a lack of availability of effective antiviral therapies and vaccines. In this context, early detection of infection with sensitive and specific laboratory tools and the prompt clinical management of this disease is a health care priority. Although a variety of techniques are currently used for laboratory diagnosis of dengue, no single methodology satisfies the ideal requirement for both sensitivity and specificity, while also being rapid and inexpensive. Newer detection tools that can fill this acknowledged gap in dengue diagnosis are urgently required.

Keywords: dengue, virus, diagnosis, serotype, antibody, nucleic acid, PCR

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1. Introduction

Dengue is a mosquito-borne viral disease that has a wide-ranging geographical distribution throughout the tropics and subtropics. It is estimated that currently over 3.9 billion people are at risk of infection [1], with 96 million notified clinical cases per year in at least 128 countries worldwide [2]. Dengue virus (DENV) is transmitted by mosquitoes of the *Aedes* genus, primarily *Ae. aegypti*, but also *Ae. albopictus* and *Ae. polynesiensis* [3]. DENV is a member of the family *Flaviviridae* [4], which also includes the other major human pathogens West Nile, yellow fever, Zika, Japanese encephalitis, and tick-borne encephalitis viruses. Four serotypes of the virus (DENV-1 to DENV-4) have been known for many years, while a putative fifth serotype, DENV-5, was identified recently [5]. This new subtype was identified during screening tests on virus samples collected during an outbreak in Malaysia in 2007 [6].

DENV infection may result in a progressive scope of clinical sequelae, viewed traditionally as ranging from asymptomatic infection through to dengue fever (DF) and the more severe disease manifestations of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). More recently [7], the World Health Organization proposed a revised classification that categorizes infection as follows: (1) dengue; (2) dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, and increasing hematocrit with decreasing platelets); and (3) severe dengue (dengue with severe plasma leakage, severe bleeding, and/or organ failure). Asymptomatic infections are characterized by undifferentiated fever with or without rashes. In the majority of clinical cases, the initial presentation of dengue is not apparent until clear signs and symptoms develop. In turn, this delay limits the ability to make an early and accurate clinical diagnosis. Moreover, there are no specific therapies for dengue, such as anti-viral drugs. Any treatment is essentially supportive, and thus, there is a very important challenge in making an informed estimate of the severity of a patient's disease as early as is practicable. The preliminary stages of DF are often confused with other febrile tropical diseases [8], which may lead to inappropriate therapy.

Although very recently, the first licensed dengue vaccine (DENV-1 to DENV-4 chimera constructed on a yellow fever 17D backbone, recombinant, live attenuated, tetravalent virus (CYD-TDV; Dengvaxia®, Sanofi Pasteur)) has been registered for use [9], major challenges to vaccine effectiveness and long-term safety in the administered population remain. This scenario, therefore, still demands a quick and reliable diagnostic approach. While a variety of laboratory diagnostic techniques have been in use for many years, each has its limitations [10], to date no tool proving to be reliable, rapid, and cost-effective. There is, therefore, a pressing need to improve the way in which dengue is diagnosed. The overall scenario calls for the development of reliable and rapid dengue diagnostic tools.

2. Current laboratory methods for dengue diagnosis

2.1. Virus isolation

Virus isolation is considered to be the gold standard technique in dengue diagnosis [11]. Isolation is highly successful when specimens are collected in the viraemic period, which

starts 2–3 days before the onset of fever and lasts for a further 2–3 days [12]. Routinely, serum is the primary choice of sample. In addition, other types of specimen, including plasma, peripheral blood, cerebrospinal fluid, pleural fluid, and tissues of reticuloendothelial origin such as liver, spleen, lymph nodes, lung, and thymus, are of substantial importance [7, 12, 13]. Diagnostic sensitivity is dependent upon the timing of specimen collection, proper storage, and transportation. Since the DENV virus particle is heat-labile, appropriate handling and prompt delivery to the laboratory is required. When storage is warranted, a standard refrigerator temperature (~4°C) is quite appropriate in the short term, while for a longer duration, freezer temperatures of −20 or −70°C are recommended. In the latter case, they should be maintained in order to prevent thawing [13].

There are different methods available for the isolation of DENV including inoculation of specimens into mosquitoes (adults or larvae); various *in vitro* insect or mammalian cell cultures; and injection intra-cerebrally in mice [12, 14–16].

2.1.1. Specimen inoculation into mosquitoes

Inoculation of specimens into both adult and larval mosquitoes with the objective to isolate DENV is considered to be the most sensitive and specific culture technique. Generally, mosquitoes belonging to the *Toxorhynchites* genus are used for this purpose. The advantage of these over *Aedes* mosquitoes is that the former has an unusual life cycle in not being hematophagous, so may be handled with impunity. In addition, the comparatively larger size of *Toxorhynchites* facilitates easier introduction of inocula. However, the fact that between 5 and 20 mosquitoes are needed to produce results of higher sensitivity is considered a drawback of this method. Alternatively, adult male *Ae. aegypti* and *Ae. albopictus* mosquitoes are also useful for virus isolation [17, 18]. The preferred route for specimen inoculation of mosquitoes is intrathoracic. Incubation for 14 days at 32°C following inoculation precedes mosquito storage at −70°C [19]. Successful virus infection of mosquitoes is confirmed by the detection of antigen in a smear prepared by crushing of mosquito heads on a microscope slide followed by serotype-specific immunofluorescence. A preparation of thorax-abdomens can also be tested for virus by plaque assay or plaque reduction neutralization test (PRNT) using monospecific dengue virus antisera targeted against different DENV serotypes [20]. The labor-intensive process of raising mosquitoes requires arranging for a second mosquito species to serve as a food source (because *Toxorhynchites* mosquitoes are carnivorous). In addition, the requirement for facilities with capacity to rear and handle infectious *Aedes* mosquitoes while adhering to stringent health and safety protocols is beyond the capability of most laboratories and remains a major obstacle. Similarly, the need for both specialist containment and highly skilled technicians in order to perform direct mosquito inoculation has combined to make *in vitro* cell culture a preferred option [21].

2.1.2. Mosquito or mammalian cell line culture

In vitro culture of cell lines is a technique being developed as an alternative tool for virus isolation and aimed at overcoming the pitfalls involved in mosquito inoculation. However, thus far the variety of approaches to cell line culture is limited by the lower sensitivity of results obtained. Cell line cultures from *Ae. albopictus* (C6/36), *Ae. pseudoscutelaris* (AP-61,

AP-64), and *Tinissa amboinensis* (TRA-284) are different choices for routine DENV isolation [22–24]. All three of these have distinct benefits and drawbacks, so preference of use should be made on a case-by-case basis. In a comparative study, the virus isolation rate was found to be highest in TRA-284, followed by AP-61 and C6/36 [24]. In contrast, C6/36 cells were preferable for detecting infected cells by the direct fluorescent antibody test (DFAT). The utility of the C6/36 cell line method to isolate virus from tissue samples derived from fatal cases of dengue has been demonstrated [16]. Problems with AP-61 and TRA-284 include frequent cell clumping and difficulties in formation of a monolayer in a tissue culture flask. Some dengue viruses, especially wild types, do not produce cytopathic effects on these cell lines [24], which limits usage of the plaque assay and PRNT for virus quantification and virus-neutralizing antibody detection. Therefore, for specific diagnosis, immunoassays should be used.

Alternatives for isolating virus are mammalian cells such as Vero and BS-C-1 (both derived from African green monkey kidney), LLCMK2 rhesus monkey kidney, PS (porcine kidney), and BHK 21 (baby hamster kidney). While these cell cultures are used widely in the PRNT, they produce less sensitive results. PRNT is considered as the benchmark means to characterize and quantify circulating levels of anti-DENV neutralizing antibody [25–27].

2.1.3. Intra-cerebral injection in mice

The oldest and least sensitive method for virus isolation is intracerebral inoculation of suckling mice. This does, nonetheless, offer a considerable benefit in enabling evaluation of the neutrotropic characteristics of the dengue virus particle *in vivo*. In most cases, mice develop encephalitis symptoms [28]. However, virus identification is generally accomplished by immunofluorescence methods with serotype-specific anti-dengue antibodies applied to infected cells or brain tissue of mice. This is a cumbersome procedure, and the need for a high specification laboratory microscope combined with difficulties in maintaining viability of the virus in specimens limit its use on a routine basis.

2.2. Viral nucleic acid detection

DENV is a single-stranded positive-sense RNA virus of approximately 50 nm in length. The 11 kb genome of each virion encodes three structural proteins (capsid, C; precursor membrane, prM; and envelope, E) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). These non-structural proteins play roles in viral replication and assembly. Structurally, a virion consists of a nucleocapsid, enveloped by an outer glycoprotein shell and an inner lipid bilayer. Surface projections in the lipid membrane consist of E and membrane (M) glycoproteins [4].

Nucleic acid amplification tests and identification of virus antigen or antibody serve as the predominant means of detection of DENV, based on the molecular or immunological response to specified viral structural components. Commonly used methods are reverse transcription polymerase chain reaction (RT-PCR), nucleic acid sequence-based amplification (NASBA), and transcription-mediated amplification (TMA).

2.2.1. Polymerase chain reaction

Virus RNA genome detection tests are faster and considered more sensitive than virus isolation and its detection. Thoughtful design of primer sequence may be applied to not only distinguish the binary presence of the target virus but also determine detailed features of the viral genome such as serotype or polymorphisms. Type-specific primers and consensus sequences that are located in different genes of most flaviviruses including dengue, such as those for E, NS1, NS3, and NS5, have been used extensively for the detection and identification of DENV [29–32]. For a PCR run, DENV RNA should pass through a conversion step to complementary DNA (cDNA) by a process of reverse transcription (RT). Conventional PCR can be used to identify the presence or absence of a target sequence in a given sample when reaction products are visualized by agarose gel electrophoresis [33], Southern blot [34], or colorimetric enzyme-linked immunosorbent assay [35]. While easy to enact, these conventional procedures have a limited inference in comparison to more versatile PCR systems. They are considered less robust as multiple handling steps are required, which increase the risk of contamination and hence the production of false-positive results.

Modification of protocols to make use of nested primers aims to reduce non-specific detection by negating improper primer binding. In nested PCR, targets are detected by using two sets of primers involving a double process of amplification. However, the greater manipulation of the sample that this technique involves may raise the risk of contamination of the amplicon product. Multiplex PCR is an adaptation that allows simultaneous amplification and detection of multiple target amplicons, which can be used to great effect in distinguishing conditions such as viral serotype. Both methods have been found to increase the sensitivity and specificity of the test and are preferred for the diagnosis of DENV [33, 36].

Real-time PCR, also known as quantitative PCR (qPCR), is a far more robust assay format than conventional PCR, over which it has many advantages including rapidity, quantitative measurements, lower contamination rate, higher sensitivity, and specificity, and ease of standardization. Furthermore, qPCR renders post-PCR procedures such as electrophoresis or blotting unnecessary, minimizing the chance of contamination. As per conventional PCR, qPCR primer sequence can be specified to detect specific DENV to a threshold that is usually less than 100 plaque-forming units [33, 37].

With advances in diagnosis, the two-step nested RT-PCR protocol, modified to a single-step multiplex RT-PCR for detection and typing of DENV, is well established [33]. In nested PCR, targets are detected by using two sets of primers involving a double process of amplification. A nested PCR assay was developed using DENV consensus primers located in the C and prM genes that amplify a 511-bp product in a reverse transcriptase PCR followed by a nested PCR with primers specific to each DENV serotype [33]. Application of multiplex qPCR for the diagnosis of dengue has been described by various studies [36–38]. Each has concluded that this technique demonstrates superior analytical and clinical performance, as well as simpler workflow, than the heminested RT-PCR [38]. More recently, several investigators have reported on fully automatic real-time RT-PCR assays for the detection of DENV in acute-phase serum samples [39–43].

2.2.2. Nucleic acid sequence-based amplification

Nucleic acid sequence-based amplification (NASBA) is a one-step transcription-mediated isothermal process for amplifying RNA. Throughout the amplification reaction, a constant temperature of 41°C is maintained. The reaction mixture contains three enzymes: avian myeloblastosis reverse transcriptase (AMV-RT); T7 RNA polymerase; and RNase H with two short, single-stranded DNA primers. Multiple transcription of RNA copies of DNA products that are produced from the initial reverse transcription step are attributable for the exponential kinetics of NASBA. Hence, synthesized RNA pools are detected by fluorescence. These "molecular beacons" are single-stranded hairpin-shaped oligonucleotide probes showing fluorescence at the 5′ end and with a fluorescence quencher at the 3′ end. When presented in an amplification reaction with their amplified target RNA, a stable hybrid is formed [44].

Studies have found a sensitivity of 98.5% and specificity of 100% when they detected DENV with NASBA [45, 46]. The effectiveness of this method has also been described for detecting dengue virus within mosquitoes [47]. It is considered more efficient than PCR in that it bypasses the use of a thermal cycler, which makes it both cost-effective and rapid (<1 day).

2.2.3. Reverse transcription loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) is a novel PCR method that is based on the principle of a strand displacement reaction involving a stem loop structure that amplifies the target genome [48]. Its advantage over other amplification methods is the ease of monitoring of amplification; pyrophosphate ions produced as a by-product of reaction substrate deoxynucleotide triphosphates form a white precipitate of magnesium pyrophosphate that can be visualized by the naked eye, measured by turbidometer, detected by hand-held UV torch, or even following staining with SYBR green® or ethidium bromide [49, 50]. Furthermore, it takes less than one hour to yield results compared to the 3–4 h typically needed to run RT-PCR [51].

Several studies have found RT-LAMP to be a superior tool to RT-PCR in terms of sensitivity and specificity for detection and accurate differentiation of flaviviruses [49, 50, 52, 53]. Sensitivity of the tool is variously reported at 100% [49, 52, 54], with a lowest detection limit of 100 copy numbers of RNAs, while specificity ranges from 87.5 to 100% [49, 50, 52, 53].

2.3. Serological methods

2.3.1. Antigen detection

During the febrile phase of dengue illness, DENV antigens may be detected in peripheral blood leukocytes, liver [54], and lung at autopsy, and less often in the thymus, lymph nodes, skin, spleen [55], bone marrow, and serosa [56]. Immunohistochemistry and enzyme immunoassay (EIA) are useful techniques to detect dengue antigens in tissue sections (frozen, paraffin-embedded) using labeled monoclonal antibodies that are visualized with markers such as fluorescent dyes (fluorescent antibody), enzymes (immunoperoxidase and avidin-biotin enzyme) or colloidal gold. Given the inconvenience of collecting samples on which to apply these techniques, their utility has been limited for routine laboratory tests. Instead, assays

have been developed against intact virus particle and recombinant viral proteins, with NS1 and E protein detection available commercially [57, 58].

2.3.2. Non-structural proteins (NS1 and NS5)-based assay

Non-structural gene, NS1, is a highly conserved glycoprotein produced by all flaviviruses in both membrane-associated and secreted forms, and which is essential for viral replication and viability [59]. Localized to cellular organelles, this antigen is secreted abundantly in sera of patients during virus replication and the early stage of infection [58]. A unique feature of this protein is its secretion by mammalian cells as hexamers (dimer subunits only), while it is not secreted by insect cells, including those of mosquitoes. Glycosylation is believed to be an important step for protein secretion. It can be identified in peripheral blood prior to formation of antibodies, and the detection rate is higher in acute primary infection from the day of onset of fever to day 9 [60, 61]. NS1 is also a complement-fixing antigen that elicits a very strong humoral response.

Several recent studies have addressed the use of NS1 antigen and anti-NS1 antibodies as a tool for dengue diagnosis [62–65], with higher specificity for determination of homologous serotypes by serotype- and group-specific NS1 capture ELISA [62, 66], and a detection sensitivity of 1–4 ng/ml NS1 in blood [67]. This tool can be utilized to differentiate between primary and secondary dengue virus infections, as proven by the highly correlated results of NS1 serotype-specific IgG (determined by ELISA) and PRNT [43, 68]. The NS1 serotype-specific IgG ELISA worked reliably for serotyping dengue virus in convalescent phase sera from patients with primary infection and also in acute phase sera from patients with secondary infection (in which the serotype that caused the first infection would be detected), but not so with convalescent phase sera from patients with secondary infections. Different commercial assays to detect NS1 antigen are available, including Panbio dengue virus Pan-E NS1 early ELISA (Alere); dengue virus NS1 antigen ELISA (Standard Diagnostics); Platelia NS1 antigen ELISA (Bio-Rad). However, none of these have excellent sensitivity, ranging from 45 to 57% [69]. NS1 ELISA has a noted lower sensitivity in sera from patients infected with DENV-4. This may be due to possible quantitative differences in the secretion of NS1 antigen by distinct serotypes [70].

Dengue non-structural protein NS5, which plays a vital role in dengue replication, is the largest and most well conserved of the flavivirus proteins [71]. One study demonstrated reliable results in diagnosing and differentiating DENV from West Nile virus and St. Louis encephalitis (SLE) virus using an immunoassay designed to targeting NS5 antigen [72]. However, questions arise as to its specificity since NS5 amino acid sequence homology between West Nile, dengue, and SLE viruses is more than 75% [72], hence a greater chance that results would be affected by cross-reactivity.

2.4. Antibody detection

DENV-specific IgM antibodies start to appear after 4–5 days of primary infection and are measurable for up to 3 months [73]. IgG antibodies appear about a week after onset of fever, persist at high titres for several weeks and then decline; however, IgG may be detected for decades [74]. IgG1 and IgG3 are the subclasses that are mainly induced [75, 76]. Following

secondary infection, memory B cells are stimulated in response to secrete DENV-specific IgG that is measurable even on the first day of symptoms and the titre remains much higher compared to during primary infection. In secondary dengue, the IgM response is variable and, in some cases, even undetectable. The most commonly used antibody detection techniques for dengue diagnosis are haemagglutination inhibition (HI) and ELISA for IgM or IgG [77].

2.4.1. Hemagglutination inhibition

The hemagglutination inhibition (HI) method was a routinely utilized method before the development of the fast and easier antibody detection methods immunofluorescence assay (IFA) and ELISA. Due to its high degree of sensitivity and ease of application, HI is still used sometimes in developing countries. The test is based on the principle whereby the property of erythrocyte agglutination by dengue proteins is inhibited when dengue antibodies are present in serum at a sufficient concentration [78]. The most important practical limitations of the HI test are its poor specificity, requirement for paired samples, and the inability to distinguish dengue serotype [35]. Moreover, the test fails to differentiate dengue from the closely related Japanese encephalitis and yellow fever flaviviruses [11, 78, 79].

2.4.2. E/M-specific capture IgM and IgG ELISA

Methods of detection of dengue by capturing E/M-specific IgM and IgG antibodies are relatively efficient in terms of their higher diagnostic sensitivity, specificity, simplicity, and feasibility of use in low-resource countries where facilities for molecular diagnosis are not standardly available [80–82].

In IgM antibody capture (MAC)-ELISA, the total IgM in a patient's sera is bound by anti-μ chain-specific antibodies precoated on the microtitre plate [83]. Due to its rapid production then gradual waning after 2–3 months ELISA based on IgM detection is considered sensitive in early dengue infection, while not being suitable in cases of prolonged infection [11, 84].

E/M-specific IgG antibody capture ELISA, also called GAC-ELISA, enables measurement of IgG for up to 10 months after infection, thereby facilitating detection of recent and past dengue infection. In general, IgG ELISA lacks specificity within the flavivirus sero-complex groups; however, the IgG response to prM is specific to individual flaviviruses [85]. Dengue secondary infection progresses to severe dengue, so it is important to differentiate secondary from primary infection. IgG avidity ELISAs can be used to determine whether an infection is primary or secondary [86, 87]. The principle behind this method is that antibodies first synthesized after primary infection exhibit a lower affinity for antigen than do those produced later during secondary infection.

Classification of primary and secondary infections can also be defined in terms of the ratio of anti-DENV IgM to IgG antibodies. A higher IgM/IgG ratio specifies the infection as being primary, for which a ratio value of 1.2 is considered as a distinguishing cut-off. However, the most reliable way to demonstrate active infection would be a significant (fourfold or greater) rise in IgM and/or IgG antibody titres between acute and convalescent phase sera [88].

Cross-reactivity between flavivirus antigens is the major issue that lowers the specificity of ELISA. While this may be reduced by detecting E/M- and NS1-specific anti-dengue virus IgM antibodies, E/M-specific IgG elicited by different flavivirus sero-complexes are highly cross-reactive [10, 11]. In order to increase the specificity of results analysis of paired serum samples from both acute and convalescent phases is strongly recommended [10, 11].

2.5. Rapid diagnostic test

Immunochromatographic tests are developed to meet the need for rapid methods of dengue diagnosis. The value of the immunochromatographic format is its rapid attainment of a result by virtue of a color change that is visible to the naked eye within 10–15 min. Tools are based on the detection of anti-dengue IgM and IgG antibodies and of dengue antigens. A number of studies have evaluated dengue rapid diagnostic tests (RDTs) [89–93]. Commonly used RDTs are Duo IgM and IgG Rapid Test Strip (Panbio); Bioline Dengue IgG/IgM (Standard Diagnostics); VScan (Minerava); Smartcheck (GlobaleMed); Denguecheck-WB (Tulip); and Dengue IgG/IgM (Core). With acute phase samples, the diagnostic accuracy of the tests has not been established reliably, but manufacturers' claimed performance ranges from 76 to 100% sensitivity and exceeding 99% specificity. In contrast, markedly different results were claimed independently [92], suggesting most RDTs are unsuitable for dengue diagnosis as they have poor sensitivity and specificity. A preliminary trial of dengue from Nepal has reported a similar finding when rapid immunochromatographic tests are comparing with ELISA; the former tool did not prove sufficiently reliable with regard to either sensitivity or specificity [82]. In order to overcome the limitations posed by RDTs, techniques have been modified whereby dengue NS1 antigen is detected in combination with anti-glycoprotein E IgM and IgG antibodies [94].

2.6. Dengue virus serotyping and genotyping

Detection and serotyping of dengue viruses are performed by molecular methods such as PCR (nested, semi-nested, and multiplex RT-PCR) using serotype-specific primers [95, 96]. Following RT-PCR, the amplified product is subjected to nucleotide sequencing. The most commonly used commercial sequencing technique for amplicon purification and sequencing is QIAquick PCR Purification Kit (Qiagen). Both strands of the PCR product are sequenced in order to avoid discrepancies. Gene sequences obtained for DENV strains are submitted to the GenBank database (http://www.ncbi.nlm.nih.gov). A basic local alignment search tool (BLAST) algorithm performed against information stored in the database finds regions of local similarity between nucleotide sequences. This may yield functional and evolutionary clues about the structure and function of any novel sequence.

3. Diagnostic limitations

A lack of sensitive, specific, rapid, and cost-effective assays remains the major hurdle to the development of diagnostic tools for dengue virus detection. Although virus isolation is

more specific, it is limited by being time-consuming and expensive, needing expertise, an inability to differentiate primary and secondary infections, and requiring acute samples (0–5 days post onset of fever) [13]. Nucleic acid (RNA) detection, although sufficiently sensitive and specific to identify both serotype and genotype in a short time is also restricted by the need for an acute sample, skilled handling, and by its incapacity to distinguish primary from secondary infections [78]. Furthermore, it is an expensive tool that is not affordable by all laboratories. While these limitations are being overcome by the detection of NS1 antigen, its sensitivity compared to genome detection methods is poor and cannot be guaranteed [68]. Tests that measure anti-dengue IgM antibodies are useful only for the first 4–6 days of infection. Also, IgM levels in secondary infection are quite low and could be below the threshold for detection. Moreover, IgG specificity is reduced due to cross-reactivity among flaviviruses [10, 11, 97].

4. Future directions

Challenges in developing a highly sensitive and specific diagnostic tool should be addressed. Possibilities for improvement lie in combining current techniques that could enhance both sensitivity and specificity. A rapid, sensitive test that combines detection of NS1, IgG, and IgM, thereby enabling diagnosis of infection throughout the course of illness, would provide a significant advancement in dengue diagnostics.

A potential tool to detect dengue is based on detection of the light transmission spectrum recorded in the near-infrared range (NIR). NIR spectroscopy is a fast, multicomponent assay that enables non-invasive, non-destructive analysis, in this case of human blood samples. NIR spectroscopy has become a widely used analytical method in the agricultural, chemical, petrochemical, pharmaceutical, and medical industries [98]. It may also find an application in the medical diagnostics field and preliminary investigations are underway. In general, optical spectroscopy is a technique that utilizes the light energy-dependent interaction with a sample (electromagnetic radiation usually quantified by wavelength). Biological substances can interact with light energy and produce a variety of optical responses—transmission, reflection scattering, and absorption. By scanning C6/36 cells either infected or not infected with DENV and/or mouse monoclonal hybridoma antibodies against DENV, differences in NIR spectra may indicate dengue infection. Analysis and standardization of these optical responses could literally help to shed light on dengue diagnosis in the future.

Considerable research has been performed to develop biosensors as effective tools for infectious disease diagnostics [99, 100]. A biosensor based on silicon nanowire can detect the 'reverse transcription polymerase chain reaction' product of dengue in less than 30 min. Peptide nucleic acid probes fixed in silicon nanowires recognize complementary DNA fragments [100]. Sensitive and specific results from these techniques could offset the other labor-intensive and time-consuming laboratory tests requiring trained laboratory staff.

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