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The Conventional Diagnostic Techniques of Dengue Fever in Endemic Areas: A Review of The Approaches with A Focus on Advanced Diagnostic Tools

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Abstract

The lack of *in vitro* and *in vivo* disease models has hampered the understanding of dengue virus pathogenesis. There are four genetically different serotypes of dengue virus (DENV), and it has long been believed that each serotype is antigenically uniform. Antigenic heterogeneity within serotypes may exist, according to recent investigations, but its origin, degree, and significance are yet unknown. Tropical and subtropical climates are home to dengue, an arthropod-borne virus that has a significant negative impact on both human health and the local economy. Dengue is the world's most common arboviral infection, with almost 4 billion people living at risk of dengue infection. A recently introduced vaccine is recommended only for seropositive individuals in a restricted age range determined by transmission intensity. Dengue control still mainly depends on vector control methods because there is currently no effective dengue vaccination for the general public or antiviral medication. The detection and successful typing of dengue virus (DENV) from patients with suspected dengue fever is important for diagnosing the disease and implementing epidemiologic control measures. Access to testing is paramount to preventing future infections and receiving adequate treatment. Dengue can be diagnosed clinically against predefined lists of signs and symptoms and by detection of NS1-based antigen testing, IgM/IgG antibody testing, and polymerase chain reaction (PCR). Furthermore, new techniques are developing that can reduce expenses and time. All around the world, these kinds of techniques can be successful in low-income and rural communities. For clinical care (i.e., early detection of severe cases, case confirmation, and differential diagnosis with other infectious diseases), surveillance, outbreak control, pathogenesis, university research, vaccine development, and clinical trials, efficient and accurate dengue diagnosis is crucial. Based on each diagnostic test's advantages and disadvantages, we describe in this overview the circumstances in which it would be most helpful epidemiologically. Serological studies frequently reveal cross-reactivity with other *flaviviruses*, though. Therefore, the creation of inexpensive, reliable, reusable, disposable, and accurate diagnostic tests is essential to preventing DENV outbreaks and reemergence in the future.

Keywords: *Dengue, NS1, Rapid Diagnostic Test (RDT), Polymerase Chain Reaction (PCR), IgM/IgG antibody testing.*

Introduction

Humans are mostly affected by the mosquito-borne disease dengue in tropical and subtropical regions of the world **[1].** It is a growing public health hazard that contributes to morbidity and mortality in suburban and urban settings **[2].** Globally, WHO has estimated that around 3 billion people reside in areas where there are risks of exposure to dengue virus, and nearly 50 million people are infected with dengue virus every year **[3].** Dengue is caused by four serotypes of dengue virus (DENV1, DENV2, DENV3, and DENV4) in the Flaviviridae family **[4].** The genomic RNA of DENV encodes three structural proteins (C, pr M, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) **[5].** At both the 5′ and 3′ ends, the DENV open reading frame is flanked by untranslated regions (5′-UTR and 3′-UTR). The DENV genome has a lot of variation in it. While

there may be antigenic variability within each serotype, little is known about the origins, frequency, and significance of this heterogeneity. DENV sequence-based typing has identified canonical clades known as "genotypes." **[6],** and each serotype has four to six geographically distinct genotypes. DENV1 comprises genotypes I, II, III (sylvatic), IV, V, and VI **[7].** DENV2 comprises Asian-I, Asian-II, Asian/American, American, cosmopolitan, and sylvatic genotypes **[8].** DENV3 comprises genotypes I, II, III, IV, and V **[9],** and DENV4 comprises genotypes I, IIA, IIB, III, and sylvatic **[10].** Specific genotypes have been associated with mild or severe disease, and heterogeneous neutralization titers suggest that the immune response to some genotypes is more cross-protective than it is to others **[11].** Most people agree that intra-serotype antigenic variation plays a role in genotype-specific case outcomes and epidemic patterns and that these intra-serotype differences are not as significant as the inter-serotype variances **[12].** Recently a fifth serotype was identified **[13].** Early diagnosis plays a crucial role in detecting an epidemic or outbreak and in undertaking effective vector control measures **[14].** Dengue infection can be diagnosed in a lab setting using a variety of techniques, including virus isolation, RNA detection, antigen assays, and antibody testing. However, viral isolation and RT-PCR-based viral RNA identification are both time-consuming processes that need specialized laboratories with expensive equipment and highly qualified staff, both of which might not be readily available in hospital settings **[14].** In most of the cases, serologic tests are used to detect IgM and IgG antibodies by ELISA. During the acute phase, the presence of IgM antibodies indicates primary infection and it appears after the viremia ends or after the fever subsides **[15].** However, in secondary infections, IgG antibodies rise to high levels within the first week of infection and reduce over 3 to 6 months **[2].** Recently, the detection of nonstructural protein 1 (NS1) antigen during the acute phase of disease in patients having primary and secondary infections has been studied in various laboratories across the world **[17].** NS1 is a highly conserved glycoprotein for all the serotypes and is produced in both cell membrane-associated and secreted forms **[16].** It is essential for virus viability or replication but has no biological activity and a precise function has not yet been assigned to it **[18].** It stimulates a strong humoral response **[19].** NS1 antigen is detectable in blood from the first day after the onset of fever up to day 9, and is also detectable in the presence of IgM antibodies and when viral RNA is negative by RT-PCR **[17].**

Conventional Methods of Laboratory Diagnostics

Serological Tests

When compared to molecular or culture-based testing, serological tests are more commonly employed to detect dengue infection since they are less expensive and easier to perform. The most popular specimens for assessing the presence of neutralizing antibodies and IgM are serum and cerebrospinal fluid. Whole blood and plasma samples are, nevertheless, sporadically utilized as test specimens. IgG antibody levels have the potential to serve as a dengue detection biomarker. After an initial infection, the human body retains its IgG level. In cases of secondary infection, it may provide equivocal findings, and in individuals infected with or immune to other *flaviviruses* (West Nile virus (WNV), Yellow fever virus (YFV), or Zika virus (ZIKV), it may show false positives.

IgM-Based Tests (IgM ELISA)

Antibodies are proteins generated by the immune system to fight against the attack of an antigen **[20].** B-cells, T-cells, and immunoglobulin proteins, such as Ig (G, M, A, E, and D), are examples of typical antibodies that are created in response to antigens. During an infection, IgM antibodies are usually produced five days after the onset of symptoms, and they persist in the human patient's body for 2–3 months, sometimes even longer **[21].** The main issue with Dengue antibody detection equipment is that samples are taken before the five-day negative window. The IgM antibody response to dengue infections is stronger in first-time cases, but the IgG antibody response is higher in secondary infections **[22].** An enzyme-linked immunosorbent assay capturing the IgM antibody (MAC-ELISA) test, first developed by the Armed Forces Research Institute of Medical Sciences, can sense IgM antibodies in dengue **[23].** Numerous commercially available MAC-ELISA testing kits have been developed and marketed to test dengue infection globally. To perform the test, the dengue IgM antibody is usually captured from the patient's serum specimen by anti-human IgM antibodies in a solid phase. A sandwich-type immunoassay is then developed using recombinant dengue-derived antigen and DENV-specific monoclonal antibody **[24].** In the United States, the Food and Drug Administration (FDA) has given clearance to the first such test named DENV Detecta IgM Capture ELISA by In Bios International, Inc. (Seattle, WA, USA) **[25].** By binding the human IgM antibody to DENV recombinant antigens in a 96-well plate, it offers qualitative detection **[26].** To verify the positive test a Plaque Reduction Neutralization

Test (PRNT) is done **[27].** To clinically prove the effectiveness of the In Bios test, the FDA conducted five clinical studies in different sites. The first clinical study showed that 2–3 days of onset fever and 4–5 days of onset fever showed only a 28.7% (2–3 days) and 40.3% (4–5) chance of a positive result. However, after 6–7 days, the percentage went up to 75.9% of a positive result, and after 8–10 days, the percentage went up to 88.7% **[28].** Another study tested the cross-reactivity of the assays. The only virus that was cross-reactive was the WNV **[29].** This assay presented a sensitivity of 96% and a specificity of 94% **[28].** These IgM fast tests and IgM-based ELISA assays have several intrinsic flaws. In clinics, the POC tests' limited sensitivity can be a major disadvantage. In the United States, only one such POC test is approved, and before six days, the test is unreliable to a certain extent. Due to some cross-reactivity, a PRNT-based test is needed to confirm the results in some cases. The incubation period for dengue fever is usually five days, and the acute illness can be observed from 0–7 days of disease infection, sometime after that period, some patients experience hemorrhagic shock. Viral load can be measured in the early stages of infection using RT-PCR or any other molecular detection technique. Simultaneously, NS1 can also be diagnosed up to 10 days of infection by the ELISA method. IgM or IgG-based testing is useful to diagnose dengue infection after a few days of infection.

IgG-Based Tests (IgG ELISA)

IgG-based tests can be used for determining past or present infections. The IgG antibody is produced after IgM, and IgG antibodies can persist for a more extended period, even lifelong **[30].** A four-fold spike in IgG antibodies is often attributed to a recent infection **[31].** IgG-based ELISA tests have been made available for purchase by Standard Diagnostics (Seoul, South Korea) and Panbio Inc. (Brisbane, Australia; now Alere Inc., Waltham, WA, USA) **[32].** The Standard Diagnostics IgG-based ELISA has a sensitivity of 81.2% and specificity of 39.8%. Panbio, on the other hand, had a sensitivity of 63.5 and a specificity of 95.3% **[32].** Other IgG ELISA tests from companies such as in bios (Seattle, WA, USA), Abcam (Cambridge, MA, USA) and Euroimmum (Lübeck, Germany) also exist **[33].** A study was done comparing these tests with their NS1 and IgM/IgG antibodies and observed that the IgG tests had a high rate of false positives and high cross-reactivity **[33].** IgG tests can be useful in certain instances, but IgM and NS1 ELISA are more useful for acute infections. Due to cross-reactivity with other flavivirus IgG antibodies, it is challenging to determine primary infection just by testing IgG-based assays **[34].**

IgM/IgG Ratio Tests

An IgM/IgG ratio test is usually a differentiating test between first-time and later infections **[35].** Optical density (OD) is measured in both IgM and IgG ELISA tests. A ratio greater than 1.32 is considered as primary infection, while a ratio below 1.32 is considered secondary **[36].** Specific laboratories often regulate the cutoff value of the OD ratio to determine the outcome of the infection. One laboratory in Northern India determined that the best cutoff ratio is 1.1 **[37].** It can be helpful to identify the type of infection present by using both IgM and IgG. The cutoff ratio is subject to change based on the results of the IgM and IgG tests.

Hemagglutination Inhibition Test

The hemagglutination Inhibition (HI) test is a standard test for distinctive primary and later DENV infections, but it cannot detect early infection **[38].** Red blood cells or tympanized human O red blood cells agglutinate due to the presence of viral antigen **[39].** Anti-dengue antibodies can prevent agglutination, and the robustness of the inhibition is tested by this test **[40].** Antibody response to a first infection is typically minimal, whereas antibody titers to subsequent infections are typically high, exceeding 1:1280. Values less than 1:1280 are typically considered to be the main infection **[40].** A study was done where an IgG ELISA and HI were tested to show if they can differentiate between primary and secondary infections **[41].** IgG ELISA divided sample OD with a mean of calibrator OD. Any value below 1 was characterized as a primary infection, and any amount over 1 was a secondary infection **[41].** The study found that HI and IgG were able to detect primary infection ideally with 16/16 samples detected. However, for secondary infections, HI was inferior to the IgG-based test. HI test was only able to detect 23/73 secondary infections, while the IgG ELISA test was able to identify 72/73 samples **[41].** The IgG ELISA test is much easier to perform than HI testing, and IgG testing is much more reliable. HI also fails to distinguish between other *flaviviruses* **[39].** However, HI tests are easier to complete than plaque reduction neutralization tests (PRNT) because the viral culture is not required for HI **[42].**

NS1 Based Tests

Nonstructural 1 (NS1) antigen plays a significant part in replicating DENV into the host cell. The antigen is produced and discharged into the bloodstream of the infected patients; as a result, it is considered an

essential biomarker for detecting flavivirus infection at an earlier stage **[43].** NS1 tests are particularly important in clinical settings because they can detect the acute phase of the DENV, and NS1 persists longer than viremia in blood **[44].** Usually, lateral flow-based rapid assays are used as rapid detection schemes, and antigen-capture ELISAs are used in laboratory-based testing **[45].** According to the CDC, the NS1-based tests show similar outcomes as molecular tests in the first week of infection **[46].** There is currently one FDA-approved NS1 test in the USA, the DENV Detect NS1 ELISA (In Bios International), and it provides qualitative results **[47].** However, currently, there are seven commercially available tests, with four of them being rapid tests **[48].** Several studies have been reported to evaluate the sensitivity and specificity performance of all these testing devices. However, the Detect NS1 ELISA (InBios International), which is the only FDA-approved product in the market, has a sensitivity of 95.9%, which makes it the superior product among its peers (89.4% by BioRad and 85.6% by Panbio) **[49].** A positive NS1 confirms a DENV infection, while a negative result does not eliminate infection possibility. If a negative test result occurs, then an IgM-based test should be performed **[46].** A notable downside of NS1-based tests is that they are not recommended after seven days **[50].**

Molecular Detection

Molecular examinations carried out in a centralized lab setting, nucleic acid tests (NATs) demand expensive equipment and knowledgeable personnel. Most NATs need RNA/DNA extraction, which is typically impossible in POC environments. With increased sensitivity and accuracy, NATs identify and measure viral RNA/DNA, enabling acute phase detection. It is possible to find the viral genome five days after symptoms appear. Numerous assays that can offer semi-quantitative, qualitative, or quantitative detection have already been created. Three fundamental processes are included in all nucleic acid detection assays: extraction and purification of the nucleic acid, nucleic acid amplification, and detection and characterization of the amplified product. Viral RNA can be extracted and purified from the samples using conventional liquid phase separation techniques (e.g., phenol, chloroform), but have been gradually replaced by silica-based commercial kits (beads or columns) that are more reproducible and faster, especially since they can be automated using robotics systems. Many labs use a nested RT-PCR technique, which involves first reverse transcription and amplification of the C/prM region of the genome using universal dengue primers, and then serotype-specific nested PCR amplification **[51].** A combination of the four serotype-specific oligonucleotide primers in a single reaction tube (one-step multiplex RT-PCR) is an interesting alternative to the nested RT-PCR **[52].** The amplification products are seen as bands with varying molecular weights on the agarose gel using ethidium bromide dye, and they are compared with conventional molecular weight markers after the products of these processes are separated by electrophoresis on an agarose gel. Dengue serotypes in this test design are distinguished by the width of their bands. Compared to virus isolation, the sensitivity of the RT-PCR methods varies from 80% to 100% and depends on the region of the genome targeted by the primers, utilized to detect or amplify the PCR products (e.g., one-step versus two-step RT-PCR), as well as the subtyping technique (e.g., restriction site-specific PCR, nested PCR, blot hybridization with specific DNA probes, sequence analysis, etc.). Non-specific amplification can lead to false positive results, thus it's critical to focus on dengue-specific genomic areas that are not shared by *flaviviruses* or other similar viruses. False-positive results may also occur as a result of contamination by amplicons from previous amplifications. This can be prevented by physical separation of different steps of the procedure and by adhering to stringent protocols for decontamination.

Real-time RT-PCR

Real-time RT-PCR is a one-step assay technique that uses customized primer pairs and probes for each dengue serotype to quantify viral RNA. Without the requirement for electrophoresis, the reaction products can be found in real-time in a specialized PCR machine thanks to the employment of a fluorescent probe. TaqMan or SYBR Green technologies have been used in the development of numerous real-time RT-PCR tests. Because the probe hybridizes specifically with the target sequence, the TaqMan real-time PCR exhibits excellent specificity. However, not all dengue virus strains may be detectable by primers and probes published in papers. This is because the sensitivity of the primers and probes is dependent on how closely they resemble the targeted gene sequence of the specific virus under study. The SYBR green real-time RT-PCR is potentially less specific but has the benefit of simple primer design and universal RT-PCR techniques. Real-time RT-PCR assays are either “singleplex” (i.e. detecting only one serotype at a time) or “multiplex” (i.e. able to identify all four serotypes from a single sample). The

multiplex assays have the advantage that a single reaction can determine all four serotypes without the potential for the introduction of contamination during the manipulation of the sample. However, the multiplex real-time RT-PCR assays, although faster, are currently less sensitive than nested RT-PCR assays. An advantage of this method is the ability to determine viral titers in a clinical sample, which may be used to study the pathogenesis of dengue disease **[53].**

Isothermal amplification methods

It is not necessary to use thermal cycling equipment for the isothermal RNA-specific amplification test known as NASBA (nucleic acid sequence-based amplification). The initial stage is reverse transcription in which the single-stranded RNA target is copied into a double-stranded DNA molecule that serves as a template for RNA transcription. Detection of the amplified RNA is accomplished either by electrochemiluminescence or in real-time with fluorescent-labeled molecular beacon probes. NASBA is a potential tool for field research on dengue infections since it has been tuned to detect dengue viruses with a sensitivity that is comparable to that of viral isolation in cell cultures. Loop-mediated amplification methods have also been described but their performance compared to other nucleic acid amplification methods is not known **[54].**

Future Tests

Numerous novel techniques for quick dengue diagnosis are presently being developed. These include isothermal PCR, electrochemical and piezoelectric detection, micro/paper fluidics, and in vivo micropatches. These technologies are all still in their infancy and will need to be further developed before they can be used as workable solutions in actual environments. We believe that a test that uses quantitative serotype-specific NS1 detection with IgM and IgG capture to distinguish between primary and secondary dengue infection would be the best approach for diagnosing dengue.

Conclusions and Recommendation

Due to the vague and inconsistent case definitions employed, the majority of dengue cases are diagnosed only based on signs and symptoms, which leads to significant uncertainty. The accuracy of each approach studied varies greatly, even when diagnostic methods are applied, depending on the methodology, kit, and epidemiological setting. This has serious ramifications since the diagnostic approach must be customized for local circumstances. Significant advancements in dengue diagnosis have been made possible by IgM capture ELISA, viral isolation in mosquito cell lines and live mosquitoes, dengue-specific monoclonal antibodies, and PCR. Nonetheless, some issues still need for the prompt creation of fresh solutions: Isolating viruses takes a lot of time. Particular lab facilities and equipment are needed for PCR, in addition to a thorough analysis of the various methods in real-world settings. The lengthy persistence of IgM antibodies and false positive reactions complicate IgM antibody detection, which calls for appropriate timing. Commercial kits still need to be carefully considered, and issues with their availability, cost, and availability as well as additional reagents need to be addressed. Effective patient care of DENV infection depends on a timely and precise laboratory diagnosis. Even while we currently possess the instruments necessary to ascertain with certainty whether a patient has a dengue infection, we are still in need of reliable predictive biomarkers that indicate the development of a severe case of the disease. Other issues and requirements exist that are not particularly connected to the advancement of technology. For example, in many countries where dengue is common, there are limitations to the laboratory infrastructure, technical experience, and research capacity. These elements hurt clinical case management, dengue surveillance, and the creation of novel dengue control strategies. It is well acknowledged that funding for dengue field operations and research is limited, notwithstanding the lack of published estimates of this support. Money needs to be raised to upgrade the infrastructure in endemic nations and boost basic public health capabilities. As a result, there may be a drop in dengue morbidity and death as well as better disease prevention, control, and awareness of DHF/DSS and dengue. As a result, more research would be done on etiology, vaccination development, and diagnosis. Lastly, to save lives, we must urgently promote the development of a suitable, quick, early, and accessible dengue diagnostic technique.

References

1. Chua, K. B., Mustafa, B., Wahab, A. A., Khairul, A. H., Kumarasamy, V., Mariam, M., ... & Rasid, A. K. (2011). A comparative evaluation of dengue diagnostic tests based on single-acute serum samples for laboratory confirmation of acute dengue. *The Malaysian journal of pathology*, *33*(1), 13.
2. José de Jesus Dias, J., Branco, M. D. R. F. C., de Sousa Queiroz, R. C., dos Santos, A. M., Moreira, E. P. B., & da Silva, M. D. S. (2017). Analysis of

dengue cases according to clinical severity, São Luís, Maranhão, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, *59*.

3. Tang, K. F., & Ooi, E. E. (2012). Diagnosis of dengue: an update. *Expert review of anti-infective therapy*, *10*(8), 895-907.
4. Lindenbach, B. D., & Rice, C. M. (2001). Flaviviridae: the viruses and their replication.
5. Henchal, E. A., & Putnak, J. R. (1990). The dengue viruses. *Clinical microbiology reviews*, *3*(4), 376-396.
6. Rico-Hesse, R. (1990). Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. *Virology*, *174*(2), 479-493.
7. Goncalvez, A. P., Escalante, A. A., Pujol, F. H., Ludert, J. E., Tovar, D., Salas, R. A., & Liprandi, F. (2002). Diversity and evolution of the envelope gene of dengue virus type 1. *Virology*, *303*(1), 110-119.
8. Añez, G., Morales-Betoulle, M. E., & Rios, M. (2011). Circulation of different lineages of dengue virus type 2 in Central America, their evolutionary time-scale and selection pressure analysis. *PloS one*, *6*(11), e27459.
9. Wittke, V., Robb, T. E., Thu, H. M., Nisalak, A., Nimmannitya, S., Kalayanrooj, S., ... & Aaskov, J. G. (2002). Extinction and rapid emergence of strains of dengue 3 virus during an interepidemic period. *Virology*, *301*(1), 148-156.
10. AbuBakar, S., Wong, P. F., & Chan, Y. F. (2002). Emergence of dengue virus type 4 genotype IIA in Malaysia. *Journal of general virology*, *83*(10), 2437-2442.
11. Gentry, M. K., Henchal, E. A., McCown, J. M., Brandt, W. E., & Dalrymple, J. M. (1982). Identification of distinct antigenic determinants on dengue-2 virus using monoclonal antibodies. *The American journal of tropical medicine and hygiene*, *31*(3 Pt 1), 548-555.
12. Bell, S. M., Katzelnick, L., & Bedford, T. (2019). Dengue genetic divergence generates within-serotype antigenic variation, but serotypes dominate evolutionary dynamics. *Elife*, *8*, e42496.
13. Lee, J., Kim, H. Y., Chong, C. K., & Song, H. O. (2015). Development and clinical evaluation of a highly accurate dengue NS1 rapid test: from the preparation of a soluble NS1 antigen to the construction of an RDT. *Diagnostic microbiology and infectious disease*, *82*(2), 128-134.
14. Shrivastava, A., Dash, P. K., Tripathi, N. K., Sahni, A. K., Gopalan, N., & Rao, P. L. (2011). Evaluation of a commercial dengue NS1 enzyme-linked immunosorbent assay for early diagnosis of dengue infection. *Indian journal of medical microbiology*, *29*(1), 51-55.
15. Halstead, S. B. (2007). Dengue. *The lancet*, *370*(9599), 1644-1652.
16. Kumarasamy, V., Chua, S. K., Hassan, Z., Wahab, A. H. A., Mohamad, M., & Chua, K. B. (2007). Evaluating the sensitivity of a commerical dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection. *Singapore medical journal*, *48*(7), 669.
17. Alcon, S., Talarmin, A., Debruyne, M., Falconar, A., Deubel, V., & Flamand, M. (2002). Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *Journal of clinical microbiology*, *40*(2), 376-381.
18. Dussart, P., Labeau, B., Lagathu, G., Louis, P., Nunes, M. R., Rodrigues, S. G., ... & Baril, L. (2006). Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. *Clinical and vaccine immunology*, *13*(11), 1185-1189.
19. Peeling, R. W., Artsob, H., Pelegrino, J. L., Buchy, P., Cardosa, M. J., Devi, S., ... & Yoksan, S. (2010). Evaluation of diagnostic tests: dengue. *Nature Reviews Microbiology*, *8*(Suppl 12), S30-S37.
20. Dwek, R. (2009). Antibodies and antigens: It's all about the numbers game. *Proceedings of the National Academy of Sciences*, *106*(7), 2087-2088.
21. Siritt, M. E. G., Halstead, S. B., Artsob, H., Buchy, P., Farrar, J., Gubler, D. J., ... & Peeling, R. W. (2010). Dengue: a continuing global threat. *Nature Reviews Microbiology*, *8*(12), S7-S16.
22. Hunsperger, E. A., Yoksan, S., Buchy, P., Nguyen, V. C., Sekaran, S. D., Enria, D. A., ... & Peeling, R. W. (2009). Evaluation of commercially available anti–dengue virus immunoglobulin M tests. *Emerging infectious diseases*, *15*(3), 436.
23. Innis, B. L., Nisalak, A., Nimmannitya, S., Kusalerdchariya, S., Chongswasdi, V., Suntayakorn, S., ... & Hoke, C. H. (1989). An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *The*

American journal of tropical medicine and hygiene, *40*(4), 418-427.

24. Porter, K. R., Widjaja, S., Darmawan, H., Lohita, Hadiwijaya, S. H., Maroef, C. N., ... & Tan, R. (1999). Evaluation of a commercially available immunoglobulin M capture enzyme-linked immunosorbent assay kit for diagnosing acute dengue infections. *Clinical Diagnostic Laboratory Immunology*, *6*(5), 741-744.
25. Adalja, A. A., Sell, T. K., Bouri, N., & Franco, C. (2012). Lessons learned during dengue outbreaks in the United States, 2001–2011. *Emerging infectious diseases*, *18*(4), 608.
26. Tsai, J. J., Liu, C. K., Tsai, W. Y., Liu, L. T., Tyson, J., Tsai, C. Y., ... & Wang, W. K. (2018). Seroprevalence of dengue virus in two districts of Kaohsiung City after the largest dengue outbreak in Taiwan since World War II. *PLoS neglected tropical diseases*, *12*(10), e0006879.
27. Kabir, M. A., Zilouchian, H., Younas, M. A., & Asghar, W. (2021). Dengue detection: advances in diagnostic tools from conventional technology to point of care. *Biosensors*, *11*(7), 206.
28. Kabir, M. A., Zilouchian, H., Younas, M. A., & Asghar, W. (2021). Dengue detection: advances in diagnostic tools from conventional technology to point of care. *Biosensors*, *11*(7), 206.
29. Kabir, M. A., Zilouchian, H., Younas, M. A., & Asghar, W. (2021). Dengue detection: advances in diagnostic tools from conventional technology to point of care. *Biosensors*, *11*(7), 206.
30. Narayan, R., Raja, S., Kumar, S., Sambasivam, M., Jagadeesan, R., Arunagiri, K., ... & Palani, G. (2016). A novel indirect ELISA for diagnosis of dengue fever. *Indian Journal of Medical Research*, *144*(1), 128-133.
31. Andries, A. C., Duong, V., Ngan, C., Ong, S., Huy, R., Sroin, K. K., ... & Buchy, P. (2012). Field evaluation and impact on clinical management of a rapid diagnostic kit that detects dengue NS1, IgM and IgG. *PLoS neglected tropical diseases*, *6*(12), e1993.
32. Blacksell, S. D., Jarman, R. G., Gibbons, R. V., Tanganuchitcharnchai, A., Mammen Jr, M. P., Nisalak, A., ... & Lalloo, D. G. (2012). Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection. *Clinical and Vaccine Immunology*, *19*(5), 804-810.
33. Lee, H., Ryu, J. H., Park, H. S., Park, K. H., Bae, H., Yun, S., ... & Lee, J. (2019). First Case of Aureimonas altamirensis Bacteremia in Korea. *Annals of Laboratory Medicine*, *39*(6), 566-571.
34. Calvert, A. E., Boroughs, K. L., Laven, J., Stovall, J. L., Luy, B. E., Kosoy, O. I., & Huang, C. Y. H. (2018). Incorporation of IgG depletion in a neutralization assay facilitates differential diagnosis of Zika and dengue in secondary flavivirus infection cases. *Journal of clinical microbiology*, *56*(6), 10-1128.
35. Prince, H. E., Yeh, C., & Lapé-Nixon, M. (2011). Utility of IgM/IgG ratio and IgG avidity for distinguishing primary and secondary dengue virus infections using sera collected more than 30 days after disease onset. *Clinical and Vaccine Immunology*, *18*(11), 1951-1956.
36. Kuno, G., Gomez, I., & Gubler, D. J. (1991). An ELISA procedure for the diagnosis of dengue infections. *Journal of virological methods*, *33*(1-2), 101-113.
37. Changal, K. H., Raina, A. H., Raina, A., Raina, M., Bashir, R., Latief, M., ... & Changal, Q. H. (2016). Differentiating secondary from primary dengue using IgG to IgM ratio in early dengue: an observational hospital based clinico-serological study from North India. *BMC infectious diseases*, *16*, 1-7.
38. Nguyen, T. H. T., Clapham, H. E., Phung, K. L., Nguyen, T. K., DInh, T. T., Nguyen, T. H. Q., ... & Wills, B. (2018). Methods to discriminate primary from secondary dengue during acute symptomatic infection. *BMC infectious diseases*, *18*, 1-10.
39. Tirosh-Levy, S., Gottlieb, Y., & Steinman, A. (2020). Stress conditions do not affect Theileria equi parasitemia levels in sub-clinically infected horses. *Ticks and tick-borne diseases*, *11*(3), 101384.
40. World Health Organization, Special Programme for Research, Training in Tropical Diseases, World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization. Epidemic, & Pandemic Alert. (2009). *Dengue: guidelines for diagnosis, treatment, prevention and control*. World Health Organization.
41. Lukman, N., Salim, G., Kosasih, H., Susanto, N. H., Parwati, I., Fitri, S., ... & Williams, M. (2016). Comparison of the hemagglutination inhibition test and IgG ELISA in categorizing primary and secondary dengue infections based on the plaque reduction neutralization test. *BioMed*

research international, *2016*(1), 5253842.

42. Kao C.L., King C.C., Chao D.Y., Wu H.L., Chang G.J. Laboratory diagnosis of dengue virus infection: Current and future perspectives in clinical diagnosis and public health. J. Microbiol. Immunol. Infect. 2005; 38:5–16.
43. Chan, H. B. Y., How, C. H., & Ng, C. W. M. (2017). Definitive tests for dengue fever: when and which should I use?. *Singapore medical journal*, *58*(11), 632.
44. Suzuki, K., Nakayama, E. E., Saito, A., Egawa, A., Sato, T., Phadungsombat, J., ... & Shioda, T. (2019). Evaluation of novel rapid detection kits for dengue virus NS1 antigen in Dhaka, Bangladesh, in 2017. *Virology journal*, *16*, 1-15.
45. Kumarasamy, V., Wahab, A. A., Chua, S. K., Hassan, Z., Mohamad, M., & Chua, K. B. (2007). Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *Journal of virological methods*, *140*(1-2), 75-79.
46. Alcon, S., Talarmin, A., Debruyne, M., Falconar, A., Deubel, V., & Flamand, M. (2002). Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *Journal of clinical microbiology*, *40*(2), 376-381.
47. Pal, S., Dauner, A. L., Mitra, I., Forshey, B. M., Garcia, P., Morrison, A. C., ... & Wu, S. J. L. (2014). Evaluation of dengue NS1 antigen rapid tests and ELISA kits using clinical samples. *PloS one*, *9*(11), e113411.
48. Trojanek, M., Maixner, J., Sojkova, N., Rohacova, H., & Stejskal, F. (2016). Evaluation of dengue NS1 antigen rapid diagnostic test. *International Journal of Infectious Diseases*, *53*, 161.
49. Anderson, N. W., Jespersen, D. J., Rollins, L., Seaton, B., Prince, H. E., & Theel, E. S. (2014). Detection of the dengue virus NS1 antigen using an enzyme immunoassay. *Diagnostic microbiology and infectious disease*, *79*(2), 194-197.
50. Martínez-Cuellar, C., Lovera, D., Galeano, F., Gatti, L., & Arbo, A. (2020). Non-structural protein 1 (NS1) of dengue virus detection correlates with severity in primary but not in secondary dengue infection. *Journal of Clinical Virology*, *124*, 104259.
51. RS, L. (1992). Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol*, *30*(3), 545-551.
52. Harris, E., Roberts, T. G., Smith, L., Selle, J., Kramer, L. D., Valle, S., ... & Balmaseda, A. (1998). Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. *Journal of clinical microbiology*, *36*(9), 2634-2639.
53. Vaughn, D. W., Green, S., Kalayanarooj, S., Innis, B. L., Nimmannitya, S., Suntayakorn, S., ... & Nisalak, A. (2000). Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *The Journal of infectious diseases*, *181*(1), 2-9.
54. Parida, M., Horioke, K., Ishida, H., Dash, P. K., Saxena, P., Jana, A. M., ... & Morita, K. (2005). Rapid detection and differentiation of dengue virus serotypes by a real-time reverse transcription-loop-mediated isothermal amplification assay. *Journal of clinical microbiology*, *43*(6), 2895-2903.