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Original Research Article

Advances in Diagnostic Technologies for Early Detection of Dengue Virus

Amit Kumar[1*], Pratulya Nandan[2]

[1]Post Graduate, Department of Microbiology, Patna Medical College and Hospital, Patna, Bihar, India
[2]Professor, Department of Microbiology, Patna Medical College and Hospital, Patna, Bihar, India

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Corresponding Author: Dr Pratulya Nandan
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Abstract:

Polymerase Chain Reaction (PCR) methods, IgM/IgG antibody tests, and NS1-based antigen screening are among the current standard approaches used for Dengue Virus (DENV) testing. However, new diagnostic tools are appearing, showing promise for reducing costs and speeding up testing processes; this is excellent news for rural and low-resource areas throughout the world. Modern tools for diagnosis have come a long way, covering both traditional and point-of-care options. Firstly, different methods have been developed and created to find other signs and variants of the Dengue virus. These include visual, electrical, microfluidic; enzyme-linked immunosorbent assays (ELISA) and biological sensors that can be used on smartphones. Recent progress in genetic and point-of-care tests, along with new technologies like personal sensors, mobile phones, and low-power electronic devices, has opened up new ways to treat diseases, to keep an eye on patients, and keep an eye on dengue in real time. In addition, there are a number of promising new quick and point-of-care medical technologies that improve innovative tools for diagnosis in this field. The development and improvement of diagnostic tools are constantly being reviewed and improved. In conclusion, newly developed methods and technologies are continually improving early detection capacities and managing the disease in the field of DENV identification, which is experiencing fast evolution. This discovery is of utmost importance in the fight against the spread of DENV and for the successful treatment of those who have contracted the virus.

Keywords: Dengue Virus (DENV), Early Detection, Diagnostic Technologies, Point-of-Care (POC) Diagnostics, Biomarkers and Serotypes.

Introduction

Currently, Dengue Virus (DENV) testing incorporates a range of traditional procedures, including NS1-based antigen detection, IgM/IgG antibody tests, and Polymerase Chain Reaction (PCR) techniques. However, new diagnostic tools are appearing, showing promise for reducing costs and speeding up diagnostic processes; this is excellent news for remote and low-resource areas throughout the world. From more traditional methods to more modern point-of-care remedies, tools for diagnosis have come a long way.

A wide range of techniques, including optical, electrochemical in nature, microfluidic, immunosorbent, enzyme-linked immunosorbent assay (ELISA), and smartphone-based biosensors, have been developed for the detection of Dengue virus indicators and serotypes [3]. New possibilities for managing diseases, clinical tracking, and dengue real-time tracking have emerged as a result of recent advances in genetic and point-of-care testing, as well as technical advancements like wireless networking, low-power electronic devices, and worn sensors. Additionally, there is potential for the advancement of intelligent diagnostic instruments in this field from a variety of newly developed quick and point-of-care tests [4]. The development and improvement of diagnostic tools are constantly being reviewed and improved. To summarize, new technologies and approaches are continually being developed in the field of DENV detection to enhance early detection capacities and manage the disease. Such advancements are of utmost importance in the fight against DENV transmission and for the successful therapy of affected individuals [4].

An estimated 390 million population experiences dengue fever every year and 2.5 billion individuals are in danger. Vectors spread this flavivirus. The key to reducing infections caused by dengue and making sure people get the treatment they need is early diagnosis. Polymerase Chain Reaction (PCR), IgM/IgG antibody experiments, and NS1-based antigen screening are the traditional techniques for detecting dengue virus. Traditional approaches, however, may be expensive and time-consuming, reducing their usefulness in economically disadvantaged and rural regions. Testing tools for the early

identification of dengue fever have come a long way in the last few years. Biosensors based on smartphones, as well as those that use light, electricity, microfluidics, and immunosorbent assays based on enzymes (ELISAs), are among these developments. Further, advancements in mobile communication, low-power applications, electronic devices, portable sensors, biochemical and point-of-care (POC) testing, dengue leadership, clinical tracking, and real-time surveillance have created new opportunities [3]. In areas where dengue fever is most prevalent, these novel innovations mean lifesaving diagnostic instruments that are easy to use, inexpensive, and quick to provide results. While these developments seem promising, there are still obstacles to be solved, such as the requirement for additional confirmation, standardization, and scaling [4].

Infecting around 390 million people each year, the Dengue virus, which is a flavivirus that is spread by vectors, poses a threat to approximately 2.5 billion individuals all over the globe1. The key to preventing further infections and making sure the right treatments are administered is a prompt diagnosis of the Dengue virus. Polymerase Chain Reaction (PCR) techniques, IgM/IgG antibody tests, and NS1 antigen testing have traditionally been the mainstays of dengue virus detection methods1. Unfortunately, rural and financially disadvantaged communities have limits when it comes to these established processes because of how laborious and expensive they are.

Recent advances in diagnostic technology have significantly transformed the early identification of Dengue virus. New biological sensors that utilize electrochemistry, microfluidics, enzyme-linked immunosorbent test (ELISA), and smartphones have emerged; these are notable developments [2]. Also, new possibilities for Dengue control, clinical tracking, and continuous surveillance have emerged thanks to advancements in wireless communications, low-power electronic components, portable sensors, and POC (point-of-care) testing [3]. Particularly helpful for areas severely affected by the Dengue virus, these innovative innovations aim to provide quick, affordable, and readily available testing tools. However, further validation, standardization, and scalability are necessary obstacles that must be overcome for these breakthroughs to realize their immense potential [4].

A rapid and accurate diagnostic test is essential for the proper treatment of individuals infected with the virus that causes dengue fever because of the wide variety of symptoms that may accompany the illness5. Immediate action must be taken in all cases of believed dengue fever in order to prevent shock, problems due to a rise in vascular leaking plasma, and possible organ damage, regardless of the results of diagnostic tests6. Because dengue infection may advance to severe and sometimes fatal complications such as hemorrhagic fever and shock, early diagnosis is crucial in stopping the disease's course [5]. The rapid and precise detection of Dengue Virus in serum or blood samples taken during the acute phase of a suspected case might have significant consequences for both the treatment of individual patients and the prevention of the spread of the illness in the general population [7]. In endemic locations, early identification is crucial for restricting the spread of viruses, especially in the early stages of an epidemic [8]. When it comes to early-stage dengue fever, the gold standard for measuring the viral load is the identification of genomic genetic material in infected persons [1].

A rapid and accurate laboratory evaluation is essential for the proper treatment of individuals infected with the virus that causes dengue fever because of the wide variety of symptoms that may accompany the illness5. Immediate action must be taken in all cases of feared dengue fever in order to prevent shock, problems due to elevated blood vessel permeability, loss of plasma, and possible organ damage, regardless of the results of diagnostic tests [6]. Because the infection from dengue may advance to severe and sometimes fatal complications such as hemorrhagic fever or shock, early diagnosis is crucial in stopping the disease's course [5]. The rapid and precise detection of Dengue Viruses in serum or blood samples taken during the acute stage of a possible infection might have significant consequences for both the treatment of specific patients and the prevention of the spread of the illness in the general population [7]. In epidemic locations, early identification is crucial for restricting the spread of viruses, especially in the early stages of an epidemic [8]. The gold standard for early-stage dengue infection diagnosis is viral load quantification by identifying genomic nucleic acids in those with the infection [1].

Epidemiology and Global Impact of Dengue Virus: Humans are particularly vulnerable to dengue fever, a virus spread by infected bites from mosquitoes. According to estimates, 100-400 million instances of dengue illness occur each year, affecting almost half of the global population [9]. Urban and semi-urban settings are the most common habitats for this illness, which is most prevalent in tropical and subtropical climates [9]. A substantial increase in dengue cases has been seen worldwide throughout the last 20 years. From 505,430 in 2000 to an unprecedented 5.2 million in 2019, the number of cases recorded by the World Health Organization (also known as the WHO) skyrocketed [10]. Mainly, dengue cases saw a dramatic jump in 2023, along with the number and severity of epidemics increasing dramatically. Dengue has a more significant effect and is more pervasive than previously thought since these epidemics have extended to areas that were formerly untouched [10].

Impact in India: With over 34% of the world's dengue cases, India is clearly a significant player when

it comes to dengue illness. Dengue fever is a real possibility for almost two-thirds of the people of India, according to estimates34. Since the middle of the 1990s, India has been seeing an increase in the incidence of dengue sicknesses, especially inside metropolitan areas. These outbreaks have gradually spread to geographical regions that were not previously impacted by the spread of the disease [11]. Official statistics show that 94,198 instances and 91 deaths were attributable to dengue fever in India in 2023 [12]. In endemic nations like India, the development of dengue strains may be significantly impacted by the prevalence of previous illnesses, revealing intricate patterns of viral progression [13].

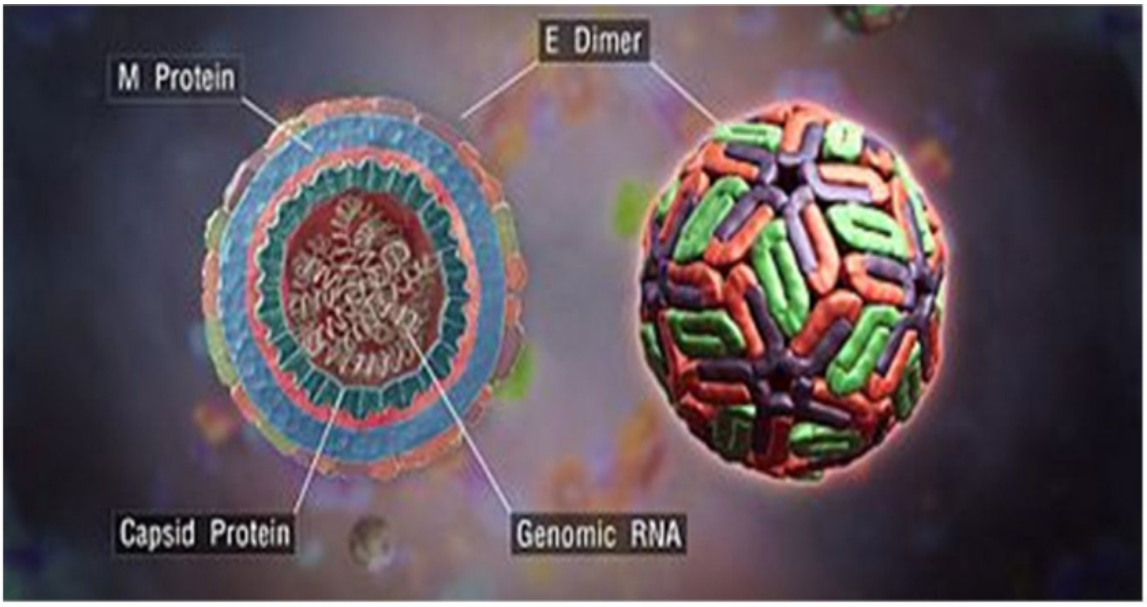

Figure 1: shows the structure of dengue virus [36]

Material and Methods:

A. Clinical Diagnosis and Symptoms: Dengue fever, which is transmitted by mosquitoes, is characterized by a rapid escalation in body temperature, accompanied by an intense migraine that often appears behind the eyes, aching muscles and joints, hives on the skin, extreme exhaustion, vomiting, and small amounts of bleeding [14].

B. Laboratory-based Methods:

1. **Polymerase Chain Reaction (PCR):** This test identifies the virus present in the collected sample [14].
2. **Blood Test:** The presence of antibodies against the infectious agent is what this test is all about. Typically, such antibodies are generated between five and seven days following a getting ill [14].
3. **Viral Isolation**: Dengue fever may be diagnosed using this time-honoured method [14,15]. The most reliable way to diagnose dengue fever is using a virus culture. Dengue diagnosis viral culture techniques include the following: One approach is to introduce a virus into mosquitoes and then watch how it develops.
4. A number of cell lines have been successfully grown in vitro with the virus. Because it is feasible, this strategy is employed more often. One approach is to introduce the infectious agent intracerebrally into the neural systems of mice.

   Ethical and practical concerns, nevertheless, make its usage less widespread. Immunoassays may be performed on serum, plasma, or white blood cells to uncover dengue viruses.

   Furthermore, it may be extracted from several post-mortem materials, including the following: thymus, cerebrospinal fluid, lymphatic system, and the spleen, the liver and pleural/ascitic fluid [15].
5. **Enzyme-Linked Immunosorbent Assay (ELISA):** Antibodies in the bloodstream may be detected and measured by this test. You may use it to find out whether you have antibodies for particular diseases[14].
6. **Complete Blood Count (CBC):** A disease in the human system that causes alterations in the quantity of leukocytes and platelets. Typically, their quantity is significantly reduced [14].
7. **Nucleic acid amplification:** A persisting acute infection [16] is indicated by the detection of DENV by use of nucleic acid multiplication by means of RT-PCR. Among the many advantages of this approach are its capacity as a quantifiable test, its ability to differentiate between DENV serotypes, and its higher

specificity when combined with real-time technology [16]. But, the RT-PCR test is not widely used since it is expensive, requires specialized machinery, and requires highly trained workers. This is especially true in many poor nations [17,18,19,20].

8. **Detection of IgG Antibody:** A substitute to the HI test, Dengue-specific IgG ELISA, has recently been implemented. Indirect IgG ELISA and the Dengue IgG Captures ELISA are two of its most prominent versions. There is a robust relationship between the HI test and the IgG ELISA, which uses the same polyvalent viral antigens as the MAC-ELISA. Additionally, the IgG avidity test, which relies on the idea that antibodies first demonstrate reduced avidity to an antigen relative to those created later, may help differentiate between initial and subsequent infection with dengue in patient samples [21,22,23].

9. Fast results, simple procedures, and applicability to large-scale monitoring investigations are just a few of the benefits of the IgG ELISA. In addition, when contrasted with the HI test, this IgG-based assay displays higher specificity. The IgG-based ELISA, however, similarly to the HI test, experiences extensive interaction with other flaviviruses in circulation. Its incapacity to determine which serotype of DENV is infecting is another disadvantage [21].

10. **NS1 antigen detection:** There are two variants of the exceptionally stable Dengue virus NS1 glycoprotein: one that is attached to the cell membranes and another that is released. In the blood of individuals experiencing the first phases of dengue fever, the amounts of produced NS1 may be seen in the range of 2 to 0.04 µg/mL [25,26,27]. It is worth mentioning that a significant amount of NS1 is already floating around in the bloodstream one day after symptoms start and continues to do so until early convalescence.

11. When no IgM nor IgG antibodies of any kind have shown up in infected individuals, this quality makes NS1 a potential substitute for the initial dengue diagnosis [28].

12. **Biosensors:** The semi-disposable microfluidic biosensor developed to detect DENV serotype 329 RNA is one of the optical biosensors published. To immobilize the target RNA, this type of biosensor uses a capture probe that is connected to supermagnetic beads. To detect fluorescence, a DNA reporter probe is attached to liposomes that contain dye. The two probes combine when they come into contact with the target RNA, creating a liposome-target-bead complex that can be seen under fluorescence microscopy. There is a threshold for detection of 10 pmol/L for the target RNA quantity, which is proportional to the number of collected liposomes [29]. A comparable work produced a microfluidic biosensor that could use microscopy with fluorescence to detect both undamaged and lysed liposomes, allowing for serotype specific DENV identification.

13. **Sensor:** In contrast to previous studies that only found intact liposomes carrying dye, this one demonstrated remarkable sensitivities, with a detection limit of 0.125 nM for intact liposomes and 50 pM for lysed ones29. Dextran sulfate, an ingredient in the hybridization solution, was responsible for the speedy 20-minute experiment time. This biosensor also showed that it could detect all serotypes of DENV by using four capture probes that were unique to each serotype [30]. To increase the concentration of the desired RNA, another team of researchers developed a nanosensor that is responsive to specific serotypes and used isothermal nucleic acid sequence-based amplification (NASBA) [31].

14. **Piezoelectric biosensor:** Signals that are electrical are generated using a piezoelectric biosensor that acts as a mass-sensitive sensor that makes use of changes that are dynamically caused. Piezoelectric biosensors employ a transducer that's constructed of a piezoelectric material, such as quartz, and a bioreceptor covered using the same substance, allowing it to vibrate at a natural resonance. A fluctuating field of electricity is generated by an external potential that regulates that frequency. As a consequence of shifts in current caused by variations in the resonance frequency, the quartz crystal microbalance (QCM) is able to identify biorecognition events. An immunochip that is capable of identifying DENV NS1 and protein envelopes is one of the piezo biosensors that have been described. This immunochip was created using QCM, and two distinct immunochips were immobilized with monoclonal antibodies that target the DENV envelope protein and NS1. Furthermore, a cocktail immunochip was also made, which includes a combination of the two antibodies [32].

Table 1: Depicts different methods of detection of dengue virus:

S. No.	Techniques	Description
1.	**Viral Detection in Cell Culture**	Dengue fever may be diagnosed using this time-honoured method [34].
2.	**Serological Testing**	Here, antibodies against the infectious agent are looked for. Typically, these kinds of antibodies are generated between five and seven days following an infection35.
3.	**RNA Amplification Using Reverse Transcriptase PCR**	The presence of the virus in the specimen may be determined by this method of testing35.
4.	**Detection of Viral Antigens**	As an example, non-structural protein 1 (NS1) identification testing may be used to identify viral antigens [2,34,35].
5.	**Nucleic Acid Amplification Tests (NAATs)**	Testing in laboratories using NAATs is the gold standard for individuals suspected of having dengue virus disease [35].
6.	**IgM/IgG Immunological Assays**	The dengue virus may be identified by these tests using IgM and IgG antibodies [2,34,35].
7.	**Biosensors**	Discoveries in biosensor technology have allowed for the detection of every dengue virus strain. They work well for the early detection of dengue disease in people. Simply immersing the biosensor in a solution consisting of sodium hydroxide for five minutes will rejuvenate it4,34,35. Its capacity to provide a comparable present reaction upon exposure to a specific DNA was preserved after regeneration [1,34,35].

Dengue virus structure, morphology and architecture: The virus that causes dengue fever (DENV) has a distinctive round shape with a diameter of around 50 nm. Essential parts of fully developed DENV virions contain proteins that attach to the cell membrane, including the M and E proteins. Scientists have studied how young inside cells virions change into the adult M form throughout virion growth. X-ray crystallographic techniques have been used in many of these studies [37].

There are five different serotypes in the DENV family, DENV1–5, and they have an amino acid sequence similarity of 65–70%. One shared genetic trait across these serotypes is a genome of single-stranded RNA, or (+)-sense RNA, that ranges in length from around 10.6 to 11 kilobases. With untranslated sections on each side of the five ′ and three ′ ends, the entire genome consists of a single open-read frame (ORF) that contains around 3400 codons (Reference: 38). This open-reading frame (ORF) generates seven non-structured proteins (NS1, NS2A-B, NS3, NS4-B, and NS5) in addition to three protein structures (capsid, membrane precursor, and envelopes). Key to the viral envelope, the E protein has a rod-like shape with two similar subunits that display an icosahedral shape and a complex herring-bone-like layout of protein dimers. This complex arrangement highlights the structural characteristics that regulate the outermost layer of the protein's form and structure [37 38].

Undoubtedly, a crucial part of clinical care is the precise and rapid diagnosis of dengue fever. The most reliable ways to determine that someone has been infected with Dengue Virus (DENV) often include testing for the virus itself, its genetic material, specific antigens, antibodies, or some mix of these. It has been shown that the virus is present in the circulation and circulation hemocytes, amongst other parts of the body. Crucial ways to detect the illness during the early stages of infection include using antigen recognition techniques and techniques for extracting and purifying the virus or its genetic material. When the infection's acute phase passes, serological testing becomes the gold standard for diagnosis.

The infection might elicit different antibody responses in other people, and these responses are dependent on their immune systems. In an effort to improve patient treatment and proactively avoid this condition, a wide variety of based in laboratory diagnosis approaches have been created. The several methods used to diagnose dengue fever are examples of continuous efforts in the medical field to improve diagnosis accuracy and maximize patient outcomes [39].

Discussion and Results

Table 1 shows that there are a number of Dengue virus infection tests that demonstrate greater specificity than the SD Bioline DD IgG test alone, suggesting that there are limits to using IgG markers solely for detection. The other tests that demonstrate higher sensitivity include the SD Dengue NS1 Ag ELISA or the SD Bioline DD NS1 or IgM or IgG tests. Although the majority of tests showed reasonable specificity, the increased risk of false positive results was due to SD Bioline DD IgG alone, which showed slightly poorer specificity. There is a high likelihood that a positive outcome correctly determines an infection with Dengue virus when using the SD Bioline DD NS1 or IgM test, and a high probability that an Unfavourable outcome correctly rules

out Dengue virus getting sick when using the SD Bioline DD NS1 or IgG test, according to the highest predictive value for a negative result.

Table 2: Latest techniques for detection of dengue virus:

Techniques	Description
CRISPR-Cas	The dengue virus may now be accurately and quickly detected using CRISPR-Cas systems [40.41]. Preparation of samples, multiplication of nucleic acids, and Cas-mediated diagnostic are all components of a CRISPR/Cas1-driven catching systemn[1,40].
Microarray	Dual illnesses with two dengue viral serotypes or single-serotype infections may be detected by extremely sensitive and precise DNA microarray assaysn [1,42].
Surface Plasmon Resonance (SPR)	Both the specificity and sensitivity of SPR make it an ideal medical diagnostic tool. Its fast anti-dengue virus identification in human serum specimens is one of its usesn[1,43].
Electrochemical Detector Sensor	The virus that causes dengue fever may now be detected using electrochemical immune sensor technology. The DENV NS1 antibodies are used for immobilizing altered electrodes, which are then used as sensors. The goal of these tests is to detect the circulation of DENV NS1 antigen in specimens, whether they have been infected or selectively spiked [44,45].

With a positive outcome, the probability of disease existence is significantly increased, and with a negative result, the probability of disease lack is moderately decreased; this is in contrast to the comparatively smaller negative probability ratio shown by the SD Bioline DD IgM solo test. The chance of illness absence with a negative test was most significantly reduced by the SD Bioline DD NS1 or IgM or IgG test. The area under the curve findings showed that the SD Bioline DD NS1 or IgM and SD Bioline DD IgM or IgG tests had a superior overall discriminating ability across all tests. The diagnostic odds ratio was most significant for the SD Bioline DD NS1 or IgM test, suggesting that it performed much better than the other tests [33].

Table 2 reveals that all tests had a moderate level of sensitivity for DENV-1, better sensitivity for DENV-2 and DENV-3, and a lack of sensitivity for DENV-4 owing to inadequate data. This is based on the serotype analysis. In most cases, sensitivities peak after six days of sickness, following which they remain mild for the first five. Acute NS1 from ViroTrack Dengue and SD Bioline DD NS1 or IgM from SD Bioline both demonstrate increased sensitivity to dengue diseases. Lab-confirmed dengue patients can be more accurately diagnosed with ViroTrack Dengue Acute NS1 and SD Dengue NS1 Ag ELISA, although both lab-confirmed and probable dengue cases may be adequately diagnosed with SD Bioline DD NS1 or IgM and SD Bioline DD NS1 or IgG, respectively, by using these assays [33]. As shown in Table 3, the ViroTrack Dengue Acute NS1 and SD Dengue NS1 Ag ELISA tests are more sensitive in identifying confirmed dengue patients, with sensitivity levels of 77.0% and 81.9%, respectively. At the same time, the sensitivity (above 78%) of the SD Bioline DD NS1 or IgM or IgG tests for identifying verified and suspected dengue patients is high [33].

Conclusion

Access to comprehensive testing stands as a pivotal factor in not only preventing subsequent infections but also in ensuring timely and appropriate therapeutic interventions. Presently, a multitude of conventional techniques are available for Dengue Virus (DENV) testing, encompassing NS1-based antigen testing, evaluation of IgM/IgG antibodies, and Polymerase Chain Reaction (PCR) assays. Concurrently, innovative approaches are surfacing within the diagnostic landscape, offering the potential to streamline both the cost and time associated with testing procedures.

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