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Understanding Pathogenesis, Clinical Features, Complications, and Diagnostic Methods of Zika Virus: A Review

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Abstract

The Zika virus (ZIKV) is a mosquito-borne illness originally identified in Uganda in 1947. It remained relatively unnoticed until 2015, when many outbreaks in the Americas raised global alarms. Unlike cases earlier isolated, the fast spread of ZIKV was associated with severe health complications, such as congenital microcephaly and Guillain-Barré Syndrome (GBS), prompting the World Health Organization to declare it a Public Health Emergency of global Concern. The basic mode of transmission is through *Aedes aegypti* mosquitoes, although the virus can also spread by sexual contact, mother to child, and through blood. ZIKV infects numerous host cells and replicates within the endoplasmic reticulum, disrupting the body's immune signaling and inducing cell death. ZIKV infection typically presents mild symptoms similar to those of the flu, including fever, rash, joint pain, and conjunctivitis (also known as pink eye).

These symptoms generally develop 3 to 14 days after being bitten by an infected mosquito and typically subside within a week. However, some people remain asymptomatic. In rare instances, Zika virus infection can cause serious complications, such as Guillain-Barré syndrome or microcephaly in babies born to infected mothers, as well as other significant fetal health problems. Diagnosis of ZIKV relies on molecular tests like RT-PCR and serological tests such as ELISA. However, the sensitivity and specificity of these tests are often influenced by cross-reactive antibodies from other flaviviruses and low levels of the virus in the blood. Current advancements in rapid diagnostics have enhanced accessibility, but ensuring standardization and validation across diverse populations remains a significant challenge. This review offers a comprehensive overview of ZIKV pathogenesis, clinical features, neurological syndrome, and diagnostic methods, emphasizing the need for innovative strategies to enhance early detection and improve public health responses to future outbreaks.

Keywords: Zika virus, pathogenesis, clinical features, microcephaly, Guillain-Barré syndrome, diagnostics, flaviviruses

Introduction

In 2015, nearly 70 years after it was found in the Zika Forest of Uganda, the mosquito-borne flavivirus known as the Zika virus (ZIKV) caught the attention of the entire globe (Dick *et al*., 1952; Nelson 2024). Since there had only been 13 naturally occurring infections in people recorded before 2007, it had not been regarded as a serious hazard to human health in previous years (Paul *et al.*, 2025). The Federated States of Micronesia experienced an outbreak in 2007 that resulted in several dozen confirmed instances of patients with a rash and fever (Sawabe *et al*., 2024). In 2013, ZIKV first became connected to Guillain-Barre syndrome (GBS), an autoimmune condition affecting the peripheral nervous system (PNS), in French Polynesia (Cao-Lormeau *et al*., 2016).

Early in 2015, a significant outbreak in Brazil was thought to have been brought on by ZIKV, with many victims describing mild fever, rash, and/or arthralgia as their primary symptoms (Paul *et al*., 2025). As a result, on February 1, 2016, the World Health Organization (WHO) deemed ZIKV a Public Health Emergency of International Concern (Honein *et al*., 2019; Burciaga-Flores *et al*., 2020). Because of the rise in microcephaly cases in Brazil during this outbreak, people became worried that ZIKV could kill and have terrible effects on the developing central nervous system (CNS). Due to this outbreak, various research teams have carried out experiments to determine how ZIKV and severe prenatal defects, including microcephaly and congenital blindness, are related (Shah *et al.,* 2024). On the other hand, illness has been linked to the emergence of Guillain-Barré Syndrome, a condition that can strike anyone at any age and leave long-lasting effects in its wake (Krauer *et al*., 2017). As a result of this and the fact that more than 80% of people who get ZIKV are asymptomatic or have few symptoms (Haby *et al.*, 2018; Burciaga-Flores *et al*., 2020), people who have any of the above symptoms should be thought to have Zika fever, chikungunya, or dengue virus infections, especially if they have travelled in the last two weeks or live in a place where arbovirus transmission has been reported recently (Colombo *et al*., 2018). Laboratory evidence of recent infection with chikungunya (CHIKV), dengue (DENV), or Zika (ZIKV) is generally established by testing patient serum to detect viral nucleic acid or virus-specific immunoglobulins and neutralizing antibodies (Dallas and Tang, 2021). Proper and prompt diagnostic techniques are imperative for the decision-making system, depending on the patient's type and symptomatology (Burciaga-Flores *et al*., 2020).

Zika virus infection typically causes a mild, self-limiting febrile illness. However, many recent outbreaks have been associated with an increase in cases of Guillain-Barré syndrome (GBS) and a rise in infants born with microcephaly. Due to these complications and the rapid spread of Zika virus infections, the World Health Organization declared Zika fever a public health emergency of international concern. Therefore, understanding the transmission, clinical features, and diagnosis of Zika virus infection is essential for implementing effective prevention and control measures (Jayachandran *et al.,* 2021). Despite numerous studies on Zika virus pathogenesis and clinical presentation, there remains a lack of an integrated, up-to-date synthesis that links recent molecular findings with clinical outcomes, complications, and diagnostic innovations, especially in low-resource and Zika-endemic regions.

METHODS

The digital archives MDPI, Science Open, PLOS, PubMed, MEDLINE, Google Scholar, Research Gate, Nature Communications, EMBASE, and Cochrane were used to search the literature. Various subheadings such as "clinical manifestations," "Pathogenesis," and "diagnosis of Zika virus" were used in conjunction with the following MeSH terms: "Zika virus infection," "Pathogenesis," "complications," "microcephaly," "Zika virus future perspective," and "Zika virus diagnosis." The pathophysiology, clinical manifestations/features, complications, and diagnostic methods were included in all reviews of the literature, original studies, and case reports. The WHO, CDC, and other public health organizations' epidemiological reports were also evaluated for this article. The goal of this search technique was to locate publications that could give readers additional in-depth information about the pathophysiology, complications, and diagnosis of the Zika virus.

PATHOGENESIS

We will have a better grasp of ZIKV pathogenesis if we have a better understanding of the ZIKV life cycle. The main mosquito is the *Aedes aegypti*, which is primarily of the Aedes (Stegomyia) genus. A mature female mosquito can lay up to 200 eggs in one batch on average when taking a blood meal, and she will typically lay five batches of eggs over her lifetime (Hussain *et al*., 2018). Their lifetime egg production capacity ranges from 500 to 1000 eggs, with 500 being the minimum and 1000 being the maximum. Typically, an adult female mosquito will bite both people and animals to get blood for the development of her eggs. Humans will be impacted as the sylvatic cycle changes into the urban cycle since the Aedes mosquitoes need to bite people to draw blood (Dallas and Tang, 2021).

The infected mosquito's saliva lubricates the skin where it encounters human blood, allowing the infected Aedes mosquito with ZIKV to puncture through the victim's skin. The amount of fluid remaining in an insect's proboscis after a previous blood meal, the size of the insect's salivary glands, and the rate of viral replication all influence the likelihood of ZIKV transmission. ZIKV has proteins on its surface called envelope (E) proteins that help it attach to the host cell membrane during endocytosis (Roozitalab *et al*., 2025). The final step in viral entrance is endocytosis using endosomes. While the capsid separates in the cytoplasm, the endoplasmic reticulum releases the viral RNA, the host ribosomes connected to the endoplasmic reticulum will translate the positive-sense RNA genome, resulting in a polyprotein that is processed by proteolytic activity for the structural and non-structural proteins (Mohd Ropidi *et al.*, 2020). Once all of the parts have been properly put together, endosomal sorting complexes will move the virion from the cell to the Golgi apparatus through ERGIC (endoplasmic reticulum-Golgi intermediate compartment) (Dallas and Tang, 2021). As soon as a mature virion leaves the cell, exocytosis is used by a single ZIKV to make two copies of itself. This is because the virus takes over the host's immune system (Kumar, 2025).

Additionally, *A. aegypti* mosquitoes act as dengue and chikungunya virus vectors. The dengue virus has spread throughout parts of South America during the past 20 years, and in some regions where the Zika virus is prevalent, the seroprevalence of dengue is above 90%. Numerous studies have shown some antigenic similarities between Zika and dengue viruses. Among dengue virus serotypes, antibody-dependent enhancement (ADE) of infection is frequent. The emergence of recent outbreaks in Dengue virus hyperendemic areas may help to explain why the latest Zika virus outbreaks are linked to neurological problems. A group of researchers led by Dejnirattisai recently showed that most antibodies that reacted with the envelope protein of the dengue virus also reacted with the Zika virus (Dejnirattisai *et al*., 2016). They did this by using a panel of monoclonal antibodies that had been made against the dengue virus. They showed that the antibodies could bind to the Zika virus but were unable to kill it, instead boosted ADE49 using the human myeloid cell line U937So, being immune to the dengue virus may help the Zika virus spread and be very important to how the disease starts and progresses in people who have been infected with Zika.

Northeastern South America saw extreme meteorological conditions in 2015 as a result of a powerful El Niño event, which may have hastened the spread of the Zika virus. According to a recent study, the Zika virus and humans share a peptide, which suggests that this could be the cause of microcephaly and GBS (Zhou *et al*., 2025). There may be additional risk factors or agents related to microcephaly besides the Zika virus.

Research led by Nogueira and others employed shotgun mass spectrometry to analyze protein extracts from three brains that had tested positive for the Zika virus. They discovered peptides from the polyprotein of the BVDV virus. According to their theory, the Zika virus may not be the only etiological factor contributing to microcephaly. However, BVDV is not known to infect humans or any individuals, nor does it cause illness. Numerous cell culture reagents are known to contain it as a contaminant. Of the 25 BVDV-derived peptides identified, 24 were found to be exact copies of human proteins. Many of these proteins belonged to the ubiquitin family. Consequently, their conclusions require experimental confirmation and are of questionable significance (Duffy *et al*., 2009).

The viral envelope protein of the ZIKV binds to receptors expressed on the vulnerable cells, including DC-SIGN, AXL, Tyro3, and TIM-1, once it has entered the host. Skin fibroblasts, epidermal keratinocytes, and skin dendritic cells are among the various cell types that the ZIKV can infect (Rodrigues de Sousa *et al*., 2021). Zika appears to have a key early target in immature dendritic cells. The Toll-like receptor 3 (TLR3), RIG-I, MDA5, interferon-stimulated genes such as OAS2, ISG15, and MX1, and beta interferon 25 are all activated through this link. Like other flaviviruses, the Zika virus may cause cells that are infected cells to undergo apoptosis. This would help the virus get past the body's natural defences and increase the initial release of infectious viral particles. Zika viruses then use autophagy to boost replication (Gratton *et al*., 2019).

One study says that treating fibroblasts that are infected with the ZIKV with 3-Methyladenine (3-MA), which stops the production of autophagosomes, greatly reduces the number of virus copies in the cells (Yu *et al*., 2025). A lot of studies using mice as models have shown that autophagy may play a big role in how Zika-related primary microcephaly happens (Yu *et al*., 2025).

CLINICAL FEATURES

The clinical presentation of uncomplicated Zika virus infection has been well documented. Due to its non-specific symptoms, the infection is often not detected or misdiagnosed. The proportion of asymptomatic infections has been reported to be around 80%. However, a retrospective serosurvey in French Polynesia revealed that among patients who tested positive for the virus, about 30% of infants and 50% of adults had asymptomatic infections. These findings highlight that the strain of the virus can affect the ratio of asymptomatic to symptomatic cases in flavivirus infections infections (Baud *et al*., 2017) The incubation period for the Zika virus is 3 to 12 days (Rabe *et al.,* 2025). The 11 (26%) of the French Polynesians who donated blood and had positive RT-PCR results for ZIKV RNA got conjunctivitis, a rash, arthritis pain, or a mix of these symptoms 3 to 10 days after giving blood. Results of a survey conducted on Yap Island showed that only 19% of those infected with the ZIKV experienced symptoms (Andrade Proaño *et al*., 2020). Maculopapular rash (present in 90% of patients), fever (65%), arthritis or arthralgia (65%), non-purulent conjunctivitis (55%), myalgia (48%), headache (45%), retro-orbital discomfort (39%), edema (19%), and vomiting (10%) are the most frequent clinical symptoms (Bothra *et al*., 2021). In Yap, there were no hospitalizations related to this incident. Similar clinical signs were seen in a group of pregnant Brazilian women who had contracted the Zika virus (Dos Santos *et al*., 2025). The rash is typically pruritic and maculopapular. The fever is usually mild and only lasts a short time. Hematospermia, hearing that is temporarily dull and metallic, swelling in the hands and ankles, and bleeding under the skin are some other strange symptoms of an acute ZIKV infection. The duration of these clinical symptoms might range from four to seven days, although they are typically self-limiting. The sole distinction is that the symptoms brought on by the ZIKVs are less severe than those brought on by other arboviruses, such as Dengue and Chikungunya. As a result, it is challenging to identify Zika virus infection only based on clinical signs (Souza-Neto *et al*., 2019).

Neutropenia and thrombocytopenia are seen in the blood image of most Zika positive patients (Schaub *et al*., 2017). Zika virus infection can lead to temporary reductions in these blood cell counts. While hematological parameters are generally normal in most cases, some patients may experience transient and mild decreases in white blood cells (leukopenia), neutrophils (neutropenia), and platelets (thrombocytopenia) during the viremic phase. During the viremic phase, when the virus replicates actively in the bloodstream, these blood abnormalities are commonly observed (Schaub *et al*., 2017). In addition to neutropenia and thrombocytopenia, patients may show other hematological alterations, including lymphopenia (a reduction in lymphocytes), monocytosis (an increase in monocytes), and raised levels of specific liver enzymes and inflammatory markers (Kain *et al*., 2021).

Although most individuals present with normal hematological parameters, some may encounter temporary, mild reductions in white blood cells (leukopenia), neutrophils (neutropenia), and platelets (thrombocytopenia) during the viremic phase.

Zika virus infection has been associated with these temporary decreases in blood cell counts. From a clinical standpoint, while these blood abnormalities are typically mild and short-lived, severe thrombocytopenia may arise in certain cases, which could lead to bleeding complications. More findings suggest besides neutropenia and thrombocytopenia, patients may also exhibit other hematological changes, such as lymphopenia (reduced lymphocytes), monocytosis (increased monocytes), and elevated levels of certain liver enzymes and inflammatory markers (Kain *et al*., 2021).

NEUROLOGIC COMPLICATIONS

Numerous recent reports have linked ZIKV to an increase in cases of fetal abnormalities like microcephaly, hydranencephaly, ventriculomegaly, cerebral calcifications, abnormally formed or absent brain structures, cataracts in both eyes, eye calcifications, and hydrops fetalis during pregnancy, though this link has not been conclusively proven (Guedes *et al.,* 2018; Teixeira *et al*., 2020). Brazil reported no cases of microcephaly, and no other country has linked ZIKV infection to an increase in microcephaly occurrences. However, several nations in the Americas and Australia have also noted a correlation between a rise in GBS cases and ZIKV outbreaks (Teixeira *et al*., 2020). The correlation between an increase in GBS cases and ZIKV infection is weaker than it is for microcephaly. Local variations in the prevalence of dengue and chikungunya make it difficult to interpret any change in regional total GBS incidence owing to the ZIKV (Harris-Sagaribay *et al*., 2020).

Brazil's recent rise in microcephaly cases has been linked to a strain with Asian ancestry. Zika virus RNA has been found in the placenta, amniotic fluid, blood, and babies with microcephalic fetuses, indicating that the virus can pass through the placental barrier (Pang *et al*., 2021). Additionally, the virus has been found in the brains and retinas of fetuses with microcephaly. Despite growing clinical evidence, there is no direct experimental proof that the Zika virus causes birth defects (Harris-Sagaribay *et al*., 2020). In mice, the Zika virus causes intrauterine developmental restrictions, including symptoms of microcephaly, as recently established by Cugola and colleagues (Pang *et al*., 2021). Additionally, they demonstrated that the ZIKV causes an increase in cell death when it infects human cortical progenitor cells in vitro. Moreover, the precise process through which a virus could result in brain abnormalities is unknown. Dang *et al*. (2016) used cerebral organoids made from human embryonic stem cells to mimic the early stages of brain development in the first trimester of pregnancy to study how it works. They demonstrated that the ZIKV effectively infects organoids and reduces their overall size by activating the innate immune receptor Toll-like-Receptor 3 (TLR3) (Vue & Tang, 2021). Early in fetal development, the nervous system is a hollow tube that runs down the back of the developing embryo. Radial glia, which contain tiny processes spanning the tube's thickness, are abundant in the inner lining of this neural tube. Radial glia are the neural stem cells that make up the developing brain's founder population. Cell loss or premature differentiation is hypothesized to be the cause of microcephaly. The AXL is thought to be a virus entry receptor that is highly expressed by human radial glial cells, astrocytes, endothelial cells, and microglia in the developing human cortex, as well as progenitor cells in the developing retina (Wahaab *et al*., 2024). It was also suggested that the ZIKV enters radial glia cells with the highest AXL expression in the developing brain through hematogenous dissemination or the CSF fluid. The ZIKV can cause severe microcephaly by preferentially killing radial glia cells, the founder cell type that creates all cortical neurons (Darmuzey, 2024).

Although ZIKV was first identified in 1947, it is unclear why a potential link between the infection, microcephaly, and GBS outbreaks was not discovered earlier. It's likely that in endemic regions, girls contract the disease and develop immunity long before they reach childbearing age. Brazil's recent increase in microcephaly instances may be related to the country's huge susceptible population, which includes pregnant mothers (Dang *et al*., 2015). When flaviviruses are introduced into new niches and populations, they can look much more harmful, but as the virus spreads and becomes established, herd immunity forms, and the severity of that specific virus eventually declines. For instance, a relatively high fatality rate resulted after the 1999 introduction of the West Nile virus to North America. Researchers linked this high virulence to certain changes that improved viral reproductive fitness in bird hosts and the environment in North America (Owusu Ntim, 2025). A single nucleotide alteration that encouraged the Chikungunya virus' adaption to a different mosquito vector was the cause of the virus' rapid spread into India (Kuldeep Dhama *et al*., 2018).

An alternate theory is that the enhanced virulence and the emergence of a new spectrum of Zika sickness are the result of viral evolutionary changes, like mutations or recombination events. Recombination activities have reportedly taken place in various Zika virus strains (Alcaraz & Nieva, 2025). The same study also discovered that some Zika virus genes, including NS5 and the envelope, are subject to intense negative selection pressure. Therefore, episodes of negative selection and the absence of positive selection indicate the elimination of undesirable variation in genes with functional value. It is thought that deletions that get rid of glycosylation sites from the envelope gene happened because of negative selection. This made the Zika virus more contagious and led to an outbreak (Dang *et al*., 2016).

The chromosomal region that codes for the E protein exhibits the insertion of a few amino acids at positions 441-442. It is necessary to investigate how those newly inserted amino acids affect the virulence of the Zika virus or the interaction between hosts and viruses (Pettersson *et al*., 2018). Shrinet and colleagues (2016) recently looked at 50 Zika virus genomes and found that all the strains that caused outbreaks in 2015–2016 had different amino acid differences in their structural and non-structural proteins. They also reported the presence of distinctive motifs in the new Zika virus strains' untranslated regions (UTRs). All these hypotheses, however, require experimental confirmation (Dang *et al*., 2016).

DIAGNOSTIC TECHNIQUES

Zika virus shares approximately 55.6% amino acid sequence identity with DENV, 46.0% with YFV, 56.1% with Japanese Encephalitis virus (JEV), and 57.0% with West Nile virus (WNV) (Akeeb *et al*., 2019). Because flaviviruses are very similar, antibodies often cross-react with them because they share many conserved epitopes that can be used as a key target for human antibody responses (Chang *et al*., 2016).

Infections with the ZIKV can be found in the serum, plasma, urine, and saliva of an infected individual for the presence of the virus RNA or by testing the semen of an infected male for clinical signs and the prevalence of the vector in the area fig. 1. (Maciejewski & Pierson 2018; Vanegas *et al*., 2021). By employing cell lines and animal inoculation, the Zika virus can be isolated from mosquitoes, different patient samples, and mosquito bites to diagnose ZIKV disease (Cintra *et al.,* 2025). However, because isolation is time-consuming, difficult, and less sensitive due to low viremia levels, it is used less commonly for diagnosis. Additionally, after the first week of sickness, neutralizing antibodies and virus-specific IgM are typically present, and they may be visible in serum for up to 12 weeks. IgM antibody detection indicates the existence of a recent or acute infection which occurs in about 3 to 12 days pass during the incubation period between the mosquito bite and the development of symptoms. In about 80% of cases, the ZIKV infection is probably asymptomatic (Santos *et al*., 2019). Every human age group is susceptible to infection with the ZIKV. In addition to serum, ZIKV RNA can also be found in urine, nasopharyngeal swabs, saliva, amniotic fluid, CSF, and frozen and fixed placentas (Dunn and Graf, 2025). Zika virus resistance varies among samples, so it is necessary to understand how the virus sheds in various bodily fluids to develop a more accurate diagnostic technique and obtain the best possible sample (Giandhari *et al*., 2025).

Real-time PCR

Real-time RT-PCR is a molecular diagnostic method that identifies ZIKV RNA in bodily specimens such as urine and blood. Viral RNA is detectable in blood for a shorter duration (around 4-7 days after symptom onset), and it can be identified in urine for up to 15 days. The extended presence of the virus in urine provides a greater chance to diagnose ZIKV infection through RT-PCR, even when the infection is no longer detectable in the blood (viremic) (George & Pinsky, 2018). The majority of molecular approaches, which are based on ZIKV genome detection, target highly conserved sections such as the UTR 5' and 3' regions or fragments of the E, C, NS1, NS3m, or NS5 genes (Khan *et al*., 2025). In times of virus outbreak, the molecular reference method and standard way to diagnose ZIKV is the reverse transcription-polymerase chain reaction (RT-PCR), which is very sensitive and specific (Jorge *et al*., 2020). Nonetheless, there are some issues with its application, for instance, the amount of virus in serum (8.1–30 106 copies/mL), saliva

(0.02–90 106 copies/mL), and breast milk (0.0004–2.1 106 copies/mL) drops quickly, which is why symptoms are often mild and not reported (Zhang *et al*., 2021).

Real time -Loop-Mediated Isothermal Amplification (RT-LAMP)

Other nucleic acid amplification and detection methods, such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), have also been developed (RPA)(Hueso *et al*., 2025). Simpler methods, like isothermal and enzymatic methods, can be used to get the viral RNA. These methods can give results in minutes, without the need to treat the sample first. These benefits allow for the creation of portable diagnostic tools (Khan *et al*., 2025). The second most popular molecular technique after RT-PCR is RT-LAMP (Silva *et al*., 2025). This method has been tested for ZIKV detection by numerous research teams, who have examined how well it works with various materials like serum, urine, and saliva. Moreover, they have been testing to ensure that it is specific to ZIKV and does not exhibit cross-reactivity with other related viruses, such as DENV or Chikungunya virus (CHIKV)(Silva *et al*., 2025). The RT-LAMP test, which has the benefit of detecting RNA in samples in an average of 15 minutes, is one example of this technology that can discriminate between the virus's lineages. In 2017, Song and Mauk created a disposable cassette for ZIKV detection based on RT-LAMP technology (Zhuang *et al.,* 2020). A temperature-controlling, chemically heated device is necessary for the cassette. Results from this system's evaluation were generated in under 40 minutes, with a detection limit (LOD) of between 50 and 100 plaque-forming units (PFU)/Ml (Nguyen *et al*., 2018). Sabalza *et al*. (2018) paired the dot blot with RT-LAMP, combining Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) with a reverse dot blot (RDB) method for detecting Zika virus (ZIKV) RNA in saliva samples. This approach enabled the detection of ZIKV RNA without prior RNA purification and was implemented on a microfluidic device. The RT-LAMP reaction amplified the target RNA, and the RDB method then detected the amplified products on a DNA array (Soares *et al*., 2021). Because they are built into a microfluidic cartridge; these two tests can find up to 857 copies of RNA/mL in saliva samples in about 15 minutes (89RT-RPA is a different method that can be used in PoC. It has been tested on clinical samples like serum, whole blood, urine, and sperm, and is 100% specific and 83% sensitive for identifying different ZIKV strains. When comparing this test to RT-PCR, which requires about 60 minutes to produce the same result, it could identify 5,102 copies of RNA on average in 10 minutes (Castro *et al*., 2018; InBios, 2019).

A different study examined the outcomes of RT-PCR using serum and saliva samples. The study's findings revealed that saliva had a higher RT-PCR sensitivity than serum. While examining saliva samples did not prolong the time frame for viral RNA detection after the beginning of illness (Jorge *et al*., 2020), samples from certain patients were positive in serum but not in saliva. The fact that higher ZIKV RNA loads can be found in the urine and semen for longer periods (10–20 and 27–62 days, respectively, following the onset of symptoms) raises the possibility of an additional diagnostic method, including the detection of ZIKV RNA in other specimen types (Madere *et al.,* 2025). Real-time RT-PCR and the Immunoglobulin M antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) test are often used to find Zika virus infections because they can detect viral nucleic acid and IgM antibodies, respectively. When viral RNA is found in the serum, a conclusive diagnosis can be made. However, because viremia is typically transient, RT-PCR diagnosis has been most successful when performed within a week of the onset of clinical illness (Madere *et al*., 20).

Enzyme-Linked Immunosorbent Assay (ELISA)
ELISA-based testing is another direct method used in the acute phase. It is usually used to find the proteins NS1 or E (pE). While the NS1 protein aids in viral replication and is also exported into the extracellular space in a hexameric form, like other flaviviruses (Kribs, 2025), the E protein is found on the virus. The ELISA technique and the plaque-reduction neutralization test, respectively, detect IgM and IgG. The main drawback of employing these conventional techniques is that they take a long time and call for trained workers to conduct experiments at BSL2 facilities (Silver *et al*., 2020). BioFront Technologies Inc. just recently created the MonoTrace Zika virus NS1 ELISA kit for the precise identification of the Zika virus non-structural 1 (NS1) protein. The assay shows robust reactivity to all major Zika virus genotypes, but it does not show cross-reactivity with Dengue virus NS1. Within the first week following the onset of symptoms, IgM antibodies against the ZIKV will manifest as viremia wanes and will last for several months (Chosewood *et al.,* 2009; Singh *et al*., 2016). Therefore, MAC-ELISA will be useful for serum samples after the first week of clinical sickness, and RT-PCR will be useful for serum samples during the first week of clinical illness. Real-time PCR and MAC-ELISA in combination are therefore anticipated to have the best diagnostic yield (Singh *et al*., 2016; Loyola *et al*., 2021). The use of serological tests for the diagnosis of Zika infection is hampered by the significant cross-reactivity among members of the family of the flavivirus. For instance, dengue infection may also cause a positive MAC-ELISA for the Zika virus (Madare *et al*., 2025).

Plaque Reduction Neutralization Test (PRNT)
The plaque reduction neutralization test (PRNT), which is the most accurate way to tell the difference between antibodies to closely related viruses, can be used to confirm the MAC-ELISA results. But this test is time-consuming, expensive, involves handling live viruses, can take up to a week to complete, and calls for standardized chemicals that are sometimes unavailable and is not commonly used. Specimens that test positive for Zika virus MAC-ELISA but negative for dengue MAC-ELISA may be regarded as having

recently contracted the virus in environments where PRNT is not available. However, it is necessary to confirm the diagnostic efficacy of this method (Shrinet *et al*., 2016; Colombo *et al*., 2018).

The ZIKV Detect 2.0 is another name for the InBios, the IgM Capture ELISA kit is a test that uses the viral envelope protein as an antigen to find high-quality IgM antibodies against ZIKV (Kherkof *et al.,* 2020). Researchers found that the InBios test still had 100% sensitivity and diagnostic results that were like those of the CDC MAC-ELISAThe Euroimmun assay may not be very sensitive because ZIKV antibodies are very specific (Granger *et al*.,2017). A diagnostic test's specificity is crucial since sensitivity affects how useful it is, and low sensitivity can result in false negative results. The tests have a lot of potential for using antibodies to check for ZIKV infections. They can confirm infections faster, which could mean that PRNT confirmation tests aren't needed as often. However, more research with a broader panel of samples is still required (Dang *et al*., 2016).

In May 2019, the FDA in the USA awarded this assay marketing permission, making it the first commercial serological test to do so. The FDA permitted the second Zika diagnostic test to be marketed in the USA for the detection of ZIKV IgM antibodies, and that test was the ADVIA Centaur Zika test. The release of the LIAISON XL Zika Capture IgM Assay II marked the third and final authorization to market the Zika Capture IgM Assay II. Previously, the FDA's Emergency Use Authorization (EUA) authority has only permitted the use of these tests in an emergency. A distinctive serological test that is still covered by the FDA's EUA is Chembio Diagnostic Systems' DPP Zika IgM Assay System (Dang *et al*., 2017; USFDA, 2019). ANVISA in Brazil has also authorized the registration of several of these commercial tests to open more diagnostic options and enable better ZIKV infection surveillance. Currently, ANVISA has approved 48 tests, 36 of which are serologic assays, as shown in fig 1 (NHSA, 2019). Most of them exhibit high sensitivity and specificity. Due to the various circulating strains of ZIKV, these tests are typically not validated using samples from more than two distinct nations or regions, which restricts their widespread and universal application. There may be changes in the genotype and phenotype of different ZIKV isolates that impact how the immune system responds and, in turn, how the virus causes an antibody response (Strottmann *et al*., 2019). These tests have a favourable chance of being used in standard diagnostic laboratories if they pass a thorough clinical review.

In a study, 30 sera from patients in the Colombian Caribbean with dengue, leptospirosis, malaria, hantavirus, and chikungunya were examined (Sánchez-Lerma *et al*., 2025). Two groups of sera were analyzed to determine the test's sensitivity: those with Zika RT-qPCR positivity and those with RT-qPCR negativity but clinical suspicion of Zika. Acute patients with a positive RT-qPCR test exhibited a specificity of 23.3% (7/30), a sensitivity of 63.6%, and an IgM sensitivity of 80% in patients who had a clinical suspicion of Zika (n = 8/10). The combined sensitivity (IgM) for both groups was 71.4% (15/21) (Presser *et* *al*., 2025; Sánchez-Lerma *et al*., 2025). The goal of the study was to compare the sensitivity and specificity of three different Zika virus detection assays. One hundred serum samples from patients presenting with acute febrile symptoms were examined using a TaqMan® RT-qPCR assay that has been previously published (Balea, 2024). To compare the outcomes, the researchers employed SYBR® Green RT-qPCR and traditional PCR techniques. In all the samples that tested negative on the TaqMan® RT-qPCR assay were also negative on the SYBR® Green RT-qPCR. The only sample that tested positive on both tests was 14% (Kappa = 0.035), which also showed positive results on both traditional PCR and agarose gel electrophoresis. Variations among the ZIKV strains in circulation globally and the low viremia phase may affect the accuracy of outbreak statistics. Therefore, better tests are necessary to improve arbovirus diagnosis and surveillance (Balea, 2024).

In a prior genetic investigation utilizing nucleotide sequences generated from the NS5 gene, East African, West African, and Asian three ZIKV lineages were identified (Seabra *et al*., 2022). The viral strains in the South American and Pacific Island outbreaks share a tight genetic link, indicating that the Pacific islands may have served as the origin of the South American epidemic. The ZIKV detection strains were made by the CDC using primer/probe combinations based on the ZIKV MR766 GenBank accession number AY632535 (country: Uganda) (Cong *et al*., 2025). These strains have different sequences (Seabra *et al*., 2022). There are currently no reliable testing protocols for diagnosing antenatal and prenatal Zika virus infection (Lackritz *et al*., 2025). By using RT-PCR to detect Zika virus RNA in the amniotic fluid, congenital Zika virus infection can be identified. The sensitivity of RT-PCR in this situation, however, has not been established (Dos Santos *et al*., 2025). While RT-PCR and MAC-ELISA can be used to test cord blood at the time of delivery, it is unknown how sensitively these techniques can identify prenatal Zika virus infection (Precit and Liesman, 2024). Real-time PCR and immunohistochemistry tests can be used to identify the presence of the Zika virus in the tissues of full-term children who died shortly after birth and fetal losses (Staples, 2016). According to recommended standard charts, occipitofrontal circumference can be used to determine microcephaly (Ramos *et al*., 2022). Microcephaly in pregnant women can be detected via ultrasonography (Mengistu *et al*., 2025). Even though prenatal abnormalities like microcephaly and others may be found as early as 18 to 20 weeks of gestation, this is frequently not the case. Aside from that, ultrasonography is not a very sensitive tool for the identification of microcephaly and is dependent on clinical and technical aspects (Mengistu *et al*., 2025). Serological and molecular tests can be used to validate ultrasound results (Dos Santos *et al*., 2025).

Since most of the affected regions are primarily developing countries, the WHO sought to improve the ability of the many laboratories worldwide to test for the virus during the Zika Strategic Response Plan (Peeling *et al*., 2025). With this shared objective in mind, they created two target product profiles (TPPs), the first of which was focused on the diagnosis of active ZIKV infection (acute phase), and the second of which was more appropriately described by Chua *et al*. (2017). This emphasizes the need for diagnostic tools with a few specific qualities at the point of care (PoC), such as a limit of detection (LOD) of 50-500 copies/mL, specificity >98%, sensitivity >95-98%, affordability, and rapid results. These tools should also be able to apply to capillary blood as well as other, less invasive samples like urine, saliva, or others, and they should be ready for use. Figure 1 lists diagnostic techniques that the US Food and Drug Administration (FDA) has approved for use in emergency situations. Since these tests do not call for pre-treating the material, they are more desirable for the creation of Proof of Concept (PoC) platforms (Burciaga-Flores *et al*., 2020; Vue and Tang, 2021).

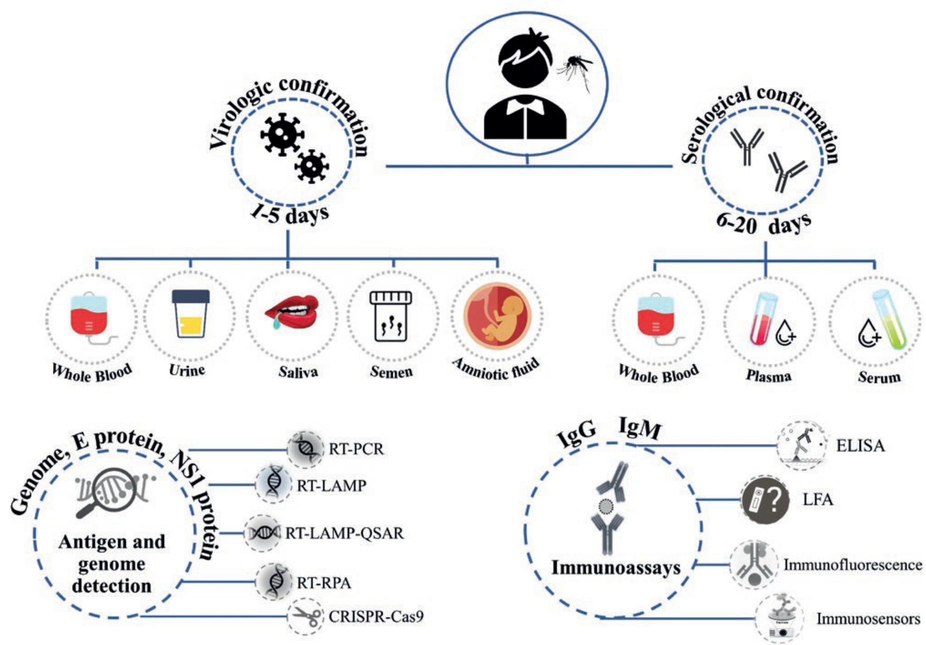

Figure 1. General diagram of detection methods for the different matrix samples. Source: Burciaga-Flores *et al*., 2020)

CONCLUSION

Undoubtedly the Zika virus has had a major impact on worldwide public health, evolving from an apparently harmless disease into a serious hazard mainly because of its strong correlation with serious neurological side effects including microcephaly and Guillain-Barré syndrome. This study highlights the complexity of ZIKV, from its varied and frequently asymptomatic clinical manifestations to its convoluted life cycle including the Aedes mosquito and its interactions with the human immune system. Uncovering the entire range of ZIKV disease requires a greater comprehension of its pathogenesis, especially the connection between ADE and dengue virus and the direct effects of the virus on brain progenitor cells.

The narrow window for viral RNA detection in some samples and the confusing problem of flavivirus cross-reactivity in serological tests are two issues that still exist despite tremendous advancements in diagnostic tool development. The necessity of constant monitoring and study is further highlighted by ZIKV's ongoing development, which may result in greater virulence.

Going forward, a multifaceted strategy is crucial. It is critical to keep funding fundamental studies of the molecular underpinnings of infection and pathogenesis of ZIKV, particularly about its neurological aftereffects. The development of quick, sensitive, and targeted point-of-care diagnostic tools that can get around the drawbacks of existing techniques and be easily implemented in environments with limited resources must be the main priority at the same time. Furthermore, for public health measures to be effective, it will be essential to comprehend the epidemiological dynamics of ZIKV, particularly the variables impacting its development and transmission. Ultimately, to lessen the current and upcoming risks presented by the Zika virus, a coordinated international effort combining research, monitoring, and creative diagnostic techniques is essential.

Recommendation and Future Considerations

With the outcome of this review, we recommend that more studies be done in different countries to unravel the pathogenesis, complications, and simpler diagnosis methods of the virus.

Although significant advancements have been made in comprehending Zika virus (ZIKV) biology and epidemiology, there are still important gaps in identification, prevention, detection, and treatment options. Future Studies should focus on the following areas:

1. *Improvement of Diagnostic Instruments* – Creating highly sensitive, affordable, and point-of-care testing methods that can differentiate ZIKV infections from other flavivirus infections, particularly in areas with limited resources where the virus is endemic.
2. *Development of Vaccines and Treatments* – Continued dedication to developing safe and effective vaccines for women of childbearing age, along with targeted antiviral therapies aimed at reducing maternal-fetal transmission and neurological complications, as well as prophylaxis.
3. *Extended Monitoring and Study of Disease Patterns* – Strengthening regional surveillance systems to track ZIKV activity, track viral changes, and understand cross-reactive immunity within both human and mosquito populations to better predict upcoming outbreaks.
4. *Study of Disease Development* – Further examinations at the molecular and cellular levels to understand the mechanisms behind ZIKV-related microcephaly,

Guillain-Barré syndrome (GBS), and other complications, to enhance prevention strategies.

5. *Coordinated Vector Management* – Developing lasting mosquito management approaches, including biological and genetic control methods, to reduce mosquito populations and decrease interactions between humans and vectors.

By concentrating on these aspects, we can strengthen our preparedness for possible future outbreaks and reduce the worldwide effects of ZIKV infections, and the associated complications.

Conflicts of Interest

All authors declared that there are no conflicts of interest.

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