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Advances in diagnosis of Dengue, Chikungunya, and Zika Virus-Infected *Aedes* Mosquitoes: A review

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Abstract
Background: Arboviral disease as Dengue, Chikungunya and Zika viruses pose significant public health challenges in India, primarily transmitted by *Aedes aegypti* and *Aedes albopictus*. While human clinical diagnosis remains standard, early detection in mosquito vectors presents a vital opportunity for proactive outbreak response and targeted vector control. However, India's current diagnostic framework exhibits several limitations—particularly in arboviral co-infection screening, differential diagnosis, DENV serotyping, and long-term infection tracking—contributing to underreporting and delayed public health interventions.
Objective: This review aims to highlight the need for robust diagnostic strategies for arboviral detection in *Aedes* mosquitoes. It underscores the importance of integrating enhanced diagnostics into surveillance programs to inform public health policy and response.
Methods: We assessed the diagnostic techniques available for DENV, CHIKV, and ZIKV, evaluating the sensitivity and specificity of available tools. Emphasis was placed on diagnostic platforms validated or applicable in the Indian context, considering resource constraints and field applicability.
Results: The review identifies several high-performance diagnostic tools suitable for use in India.
Conclusion: There is an urgent need in India to adopt enhanced, cost-effective, and scalable diagnostic tools for arboviral surveillance, particularly in mosquito vectors. Strengthening differential diagnostics, expanding DENV serotyping, and implementing systems for long-term infection tracking are essential for mitigating disease burden, guiding vector control, and improving outbreak preparedness across the country.

Keywords: Aedes, dengue virus, chikungunya virus, zika virus, diagnostics, tools

Introduction

Aedes mosquitoes have emerged as one of major public health threats due to their rapid geographic expansion, recurrent epidemics, and capacity to cause severe clinical manifestations. The species which is known to transmit DENV, CHKV, & ZIKV predominantly is Aedes aegypti and Aedes albopictus, (highly anthropophilic). According to Global estimates more than 3.9 billion people across over 129 countries are at risk of dengue alone, and ~ 390 million dengue infections occurring annually [1, 2]. Similarly, for chikungunya and Zika frequent outbreaks have been reported in many countries particularly in Asia, the Americas, and the Pacific Islands [3–5].

Rapid urbanization, unplanned settlements, inadequate water management, climate change, and increased global mobility have facilitated the spread of Aedes vectors around the globe [6]. Entomological surveillance, is an important tool especially the detection of arboviruses in mosquitoes for early outbreak warning and control. Identifying infected mosquitoes before any outbreak of disease allows timely public health responses, including targeted vector control and enhanced surveillance. This approach also improves understanding of transmission dynamics, virus-vector interactions, and seasonal risk trends [7, 8]. Recent advancements in diagnostic technologies have significantly enhanced and improved sensitivity, specificity, and field applicability. Traditional methods such as virus isolation and immunofluorescence assays, though reliable, are labor-intensive and time-consuming. In contrast, molecular techniques like reverse transcription-polymerase chain reaction (RT-PCR), real-time quantitative PCR (qPCR), and loop-mediated isothermal amplification (LAMP) have revolutionized virus detection in entomological samples by offering rapid and accurate results. Furthermore, innovations in portable diagnostics, point-of-care devices, biosensors, and next-generation sequencing (NGS) have opened new avenues for real-time surveillance, even in resource-limited settings [9–11].

This review aims to provide a comprehensive overview of the current and emerging diagnostic tools for detecting DENV, CHIKV, and ZIKV in Aedes mosquitoes. It critically examines their principles, advantages, limitations, and suitability for field versus laboratory application. Strengthening entomological surveillance through innovative diagnostic strategies is essential not only for controlling current arboviral threats but also for anticipating and mitigating future ones.

Vector and transmission

Two species of mosquitoes—Aedes aegypti and Aedes albopictus—are the primary vectors responsible for the transmission of dengue, chikungunya, and Zika viruses in humans. These vectors are highly adapted to urban environments, daytime feeding behavior, and highly anthropophillic.

The transmission cycle begins when a female mosquito feeds on a viremic host. Upon ingestion, the virus enters the mosquito's midgut then crosses midgut barriers to enter the hemocoel (mosquito's body cavity) [12]. It subsequently reaches and infects the salivary glands, the whole process takes place within 7–10 days, known as the extrinsic incubation period (EIP) of the virus within the mosquito [12,

[13] and it can be transmitted to a new human host during the mosquito's next blood meal. During feeding, the mosquito injects saliva with anticoagulants with active viral particles, which enter the dermis and bloodstream of the new human host, initiating infection [14, 15].

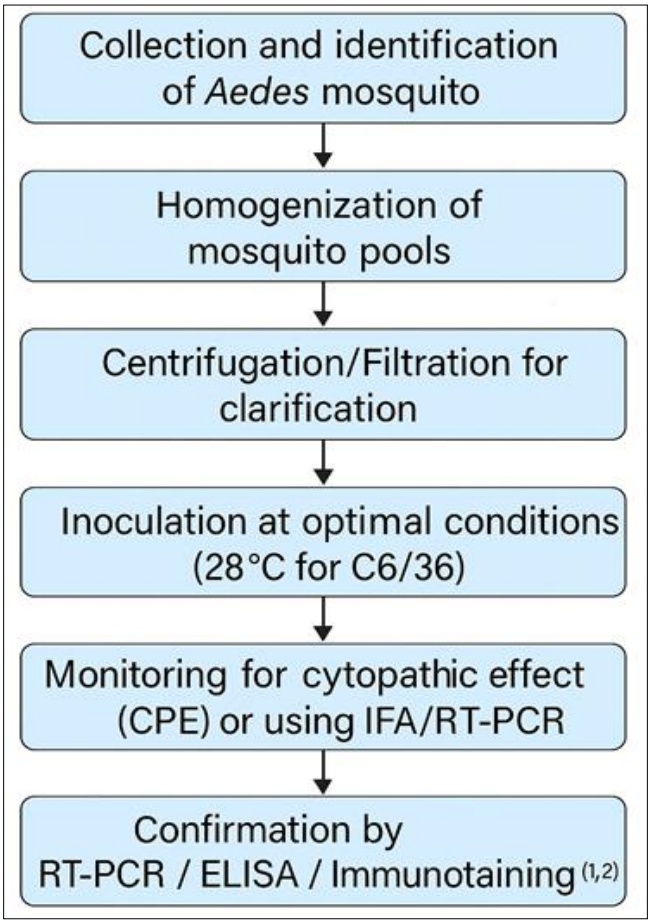
Xenomonitoring: A Tool for Early Arboviral Surveillance

Xenomonitoring is the non-invasive detection of pathogens in vector populations, such as mosquitoes, to assess transmission risks before human cases emerge. It plays a pivotal role in early warning systems, especially in arbovirus-endemic areas. By screening pooled mosquito samples for viral RNA or antigens.

Viral nucleic acids or antigens can then be detected in field-collected mosquito pools using tools such as:

Virus isolation

Virus isolation involves culturing live viruses from mosquito homogenates in susceptible cell lines under controlled laboratory conditions. When infected homogenates are inoculated into permissive cell lines (e.g., C6/36 from *Aedes albopictus*) the virus replicates within these cells. Viral replication is monitored through cytopathic effects (CPE), immunofluorescence assays, or RT-PCR from the supernatant. It confirms the presence of viable virus particles and helps in downstream applications like sequencing, antigen characterization, and vaccine research [16, 17].



Virus isolation is a highly specific method that confirms the presence of infectious virus. However, the technique has notable limitations. It is time-consuming, often requiring 5–14 days for results, and demands biosafety level 2 or 3 (BSL-2/3) laboratory infrastructure. Additionally, it is resource-intensive in terms of cost and labor, making it less suitable for rapid diagnosis, particularly in field or rural settings [18].

Immunoassays

Immunoassays detect viral antigens or host antibodies by exploiting antigen-antibody interactions. Two commonly used assays are:

1. **Enzyme-Linked Immunosorbent Assay (ELISA):** This method detects viral NS1 antigen or host-generated IgM/IgG antibodies using enzyme-linked secondary antibodies. A color change (via substrate reaction) indicates presence and quantity of antigen or antibody [19].

2. **Indirect Immunofluorescence Assay (IFA):** Viral antigen is immobilized on a slide, and mosquito sample antibodies (IgM/IgG) are detected using fluorescently-labeled secondary antibodies under a fluorescence microscope [20].

These methods are faster and less resource-intensive than cell culture but are limited by cross-reactivity, especially among Flaviviruses (e.g., DENV, ZIKV), reducing specificity [21]

ELISA	IFA
Collection of mosquito homogenate	Viral antigens fixed on glass slide
↓	↓
Microplate wells coated with Viral antigens (for IgM/IgG detection)	Add test serum/homogenate; incubate to bind antigen-specific antibodies
↓	↓
Non-specific sites blocked with protein solution (e.g., BSA).	Unbound antibodies washed off.
↓	
Add test samples to coated wells and incubate (antigen or antibody binds).	Add Fluorescent Secondary Antibody (FITC) -labeled anti-human/mouse IgM/IgG
↓	↓
Add enzyme-conjugated detection antibody (e.g., anti-IgM-HRP).	Mount slide and examine under fluorescence microscope
↓	↓
Add chromogenic substrate (e.g., TMB); color develops	Fluorescent signal indicates positive antibody-antigen binding.
↓	
Measure absorbance with ELISA reader. Intensity ∝ antigen/antibody amount.	

Immunoassays, such as ELISA, offer a rapid and practical approach for arbovirus detection, delivering results within hours and requiring only basic BSL-2 laboratory infrastructure. They are useful for both early-phase detection (e.g., NS1 antigen) and late-phase serological diagnosis (IgM/IgG). However, these assays face limitations, including cross-reactivity among flaviviruses, which can compromise specificity. Additionally, they generally have lower sensitivity compared to molecular or culture-based methods and require careful calibration for reliable use in field settings [20, 21]

RT-PCR and qRT-PCR

Reverse Transcription Polymerase Chain Reaction (RT-PCR) is a molecular technique that detects viral RNA by converting it to complementary DNA (cDNA) using reverse transcriptase, followed by amplification using polymerase chain reaction (PCR).

- **Multiplex RT-PCR:** Uses multiple primer sets in a single reaction to simultaneously detect DENV, CHIKV, and ZIKV RNA in mosquito pools or clinical samples. It offers high sensitivity, specificity, and cost-effectiveness [22, 23].
- **Quantitative Real-Time RT-PCR (qRT-PCR):** Extends RT-PCR by incorporating fluorescent dyes or probes to measure the amount of target nucleic acid in real time. It enables viral load quantification and is highly reproducible, making it the preferred method in national surveillance systems and outbreak settings [24].

Field-collected mosquitoes are pooled, identified, and homogenized
↓
Viral RNA is extracted
↓
RNA is reverse transcribed reverse transcriptase enzyme.
↓
Specific primers for DENV, CHIKV, and ZIKV amplify viral genes.
↓
For RT-PCR: Amplicons detected by agarose gel electrophoresis For qRT-PCR: Fluorescent signal monitored in real-time during amplification
↓
Interpretation Ct value (Cycle threshold) in qRT-PCR correlates with viral load. Gel bands confirm virus presence in conventional RT-PCR.

Molecular techniques, particularly real-time RT-PCR, are widely used for arbovirus detection due to their high sensitivity and specificity, even at low viral loads [22]. They offer faster results than culture-based methods and can be multiplexed to detect multiple pathogens simultaneously, saving time and resources [23]. Additionally, qRT-PCR enables viral load quantification, which is valuable for assessing infection severity and transmission risk [24]. However, these methods require costly equipment and are highly dependent on RNA quality and reagent stability. Primer or probe mismatches can also reduce sensitivity across different virus serotypes, potentially affecting diagnostic accuracy [25].

Loop-Mediated Isothermal Amplification (LAMP)

LAMP provides a cost-effective, rapid, and field-deployable method for detecting arboviruses in *Aedes* mosquito pools. LAMP assays targeting DENV and ZIKV have been field-tested using colorimetric indicators (e.g., Hydroxynaphthol blue, phenol red) and fluorescence-based dyes (e.g., SYBR Green, calcein), offering visual confirmation of results [26]. These characteristics make LAMP ideal for point-of-care diagnostics and entomological surveillance.

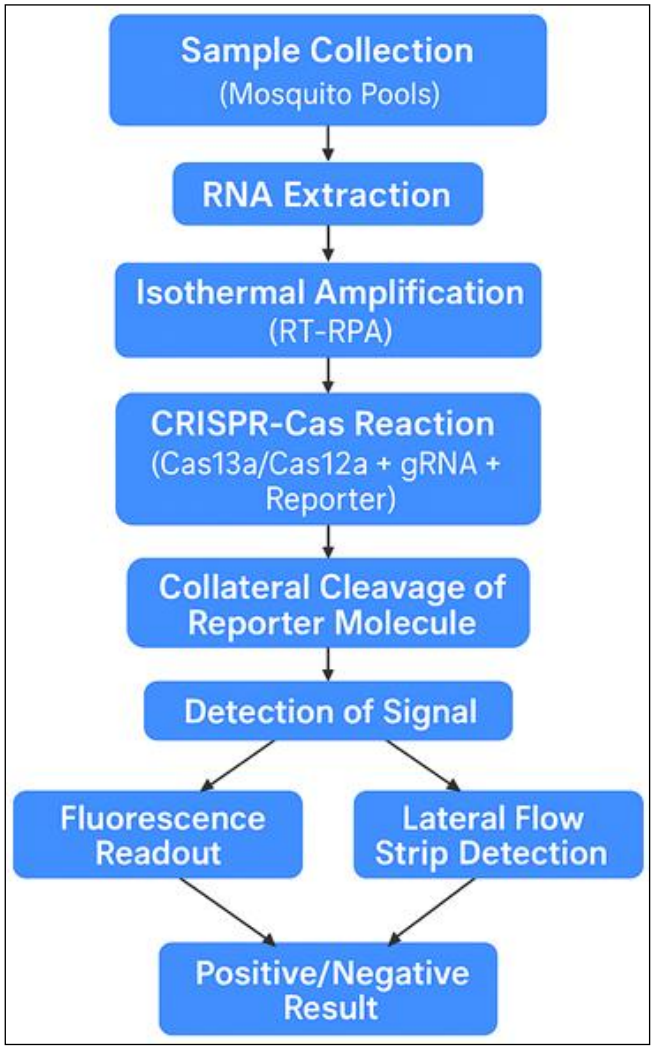
LAMP is a nucleic acid amplification technique with rapid and field-friendly molecular method for pathogen detection. It operates without the need for thermocyclers, relying on a simple, constant heat source, and delivers results within 30–60 minutes [27, 28]. LAMP offers high specificity and sensitivity and allows for easy visual detection, eliminating the need for gel electrophoresis or costly detection systems [26].

CRISPR-based Detection

CRISPR-Cas systems is highly sensitive and specific nucleic acid detection technique. The CRISPR-Cas system operates through a programmable guide RNA (gRNA) that directs a Cas enzyme to a complementary nucleic acid target. Upon target recognition

- Cas13a (in SHERLOCK) targets RNA, while
- Cas12a (in DETECTR) targets DNA, usually converted from RNA by reverse transcription.

Once activated, the enzyme exhibits collateral cleavage activity, cutting nearby reporter molecules (e.g., fluorescent- or biotin-labeled) to generate a detectable signal [29,30] . A pre-amplification step, typically RT-RPA (Reverse Transcription Recombinase Polymerase Amplification), is employed to enhance sensitivity before CRISPR-based detection [29,31].



Detection outputs can be visualized via Fluorescence measurement, or Lateral flow assay strips that display colored bands [31]. CRISPR-based diagnostics offer ultra-sensitive and highly specific detection of arboviruses, with results available in about an hour. They are equipment-free,

compatible with simple lateral flow or visual readouts, and ideal for field-based surveillance in low-resource settings.

Lateral Flow Assays (LFAs)

LFAs are immunochromatographic tests that offer a rapid, user-friendly, and field-deployable approach for the detection of viral antigens, particularly NS1 protein of Dengue virus (DENV), in mosquito pools. LFAs have also been evaluated for entomological surveillance, particularly in vector control programs for rapid screening of field-collected mosquitoes [33]. However, the sensitivity of LFAs is generally lower compared to molecular diagnostics like RT-PCR or LAMP (32,34). Rapid diagnostic tools provide results within 15–30 minutes and are highly suitable for field deployment, requiring neither electricity nor cold chain. Their simplicity makes them valuable during outbreak situations. However, these tools generally have lower sensitivity compared to molecular methods like RT-PCR, potentially missing low viral loads or early infections. Therefore, they are best used in combination with more sensitive techniques such as RT-PCR or LAMP for comprehensive surveillance and diagnosis [32, 34].

Microfluidic and Lab-on-a-Chip (LOC) Platforms

LOC platforms are miniaturized diagnostic systems that integrate multiple laboratory functions—such as nucleic acid extraction, amplification, and detection—on a single microchip. These compact, automated systems are being actively explored for field-deployable, point-of-care diagnosis of arboviruses particularly in endemic regions [36, 37].

Microfluidic systems work by manipulating fluids in channels sized from tens to hundreds of micrometers. Their diagnostic workflow typically involves

1. Sample introduction: e.g., mosquito lysate
2. RNA extraction using magnetic beads or solid-phase extraction.
3. On-chip amplification via LAMP or RT-PCR.
4. Detection through integrated sensors—optical (fluorescence), colorimetric, or electrochemical.

These chips often use capillary flow, micropumps, or microvalves to move and mix reagents, and can be operated with minimal external equipment, including smartphones for detection(38). While field-friendly, their setup may still necessitate trained personnel, especially during early implementation and are Still under development or pilot testing in many settings (36)

Portable Sequencing and PCR Devices

Technologies such as the MinION nanopore sequencer (Oxford Nanopore Technologies) and GeneXpert PCR systems (Cepheid) are currently being explored for use in endemic field sites for rapid detection, serotyping, and genomic characterization of arboviruses [39, 40]. These platforms enable faster public health responses, especially during outbreaks. MinION is a portable, USB-powered nanopore sequencer that detects nucleotide sequences by measuring ionic current changes as DNA/RNA strands pass through nanopores. It supports whole-genome sequencing, making it useful for virus tracking, lineage typing, and mutation analysis [39]. GeneXpert is a real-time PCR platform that uses disposable cartridges to extract, amplify, and detect nucleic acids with minimal user input. It can deliver automated, quantitative results in under 90 minutes and is being adapted for arboviruses [40].

Both systems are compatible with mosquito pool testing, enabling on-site, rapid surveillance of circulating arboviral strains. MinION demands technical expertise and computational resources for data analysis, while GeneXpert cartridges are expensive and often virus-specific. Additionally, both systems rely on stable electricity or battery backup, which can be challenging in remote or resource-limited settings.

Discussion

Vector-borne flaviviruses such as Dengue, Zika, and Japanese Encephalitis continue to pose significant public health threats in India, with frequent outbreaks in both urban and rural areas. Despite substantial technological advancements in diagnostic methods, several implementation challenges persist. Another significant barrier is the shortage of trained personnel and laboratory infrastructure. To overcome these limitations, India must prioritize field validation and WHO prequalification of low-cost, portable CRISPR and LAMP-based diagnostics. Moreover, integration with Geographic Information Systems (GIS) and mobile health platforms can greatly improve surveillance efforts. Innovative approaches using artificial intelligence (AI) are also under exploration in India. Such platforms could improve differential diagnosis in field settings, thereby enabling more accurate epidemiological surveillance and reducing the burden on healthcare systems during outbreaks. Taken together, the integration of conventional and novel diagnostics is essential for a tiered surveillance strategy. For example, rapid tests and LAMP assays can serve as frontline screening tools in field settings, while confirmatory testing using qRT-PCR or sequencing platforms can be centralized in regional laboratories. Furthermore, the potential of xenomonitoring as a non-invasive, early-warning surveillance method highlights the need for diagnostic tools optimized for mosquito pool testing, with sensitivity adequate to detect low viral titers [41, 42].

Future priorities should include the development of multiplexed, portable assays; improvements in assay stability under tropical conditions; and capacity-building initiatives to enable decentralized surveillance. Investment in scalable, low-cost platforms tailored to endemic regions will be crucial for timely detection, response, and containment of arboviral outbreaks. Ultimately, strengthening entomological surveillance with innovative diagnostics will enhance our preparedness for emerging vector-borne threats in an increasingly interconnected and climate-sensitive world.

Conclusion

The ability to detect DENV, CHIKV, and ZIKV in Aedes mosquitoes has improved significantly over the past two decades. While conventional techniques like virus isolation and ELISA remain relevant, molecular and CRISPR-based methods offer rapid, field-adaptable, and highly sensitive alternatives. Integration of these tools into national surveillance and vector control programs can revolutionize arbovirus outbreak forecasting and preparedness. Continued investment in innovation, validation, and capacity building is essential for wide-scale adoption and impact. Efforts towards public health, education, and resource allocation are

essential to raise awareness about these viral agents' risks and consequences, ensuring informed public policy initiatives and effective disease management that can affect global health. India faces unique challenges in the implementation of advanced flavivirus diagnostics due to infrastructural, logistical, and economic constraints. However, with strategic investment in indigenous development, workforce training, and integration of digital technologies, the country is well-positioned to lead in the development and deployment of next-generation vector-borne disease diagnostics

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