



सत्यमेव जयते
Ministry of Health & Family Welfare
Government of India



A Comprehensive Learning Guide

Zika Virus



**NATIONAL CENTRE FOR DISEASE CONTROL
DIRECTORATE GENERAL OF HEALTH SERVICES**
Ministry of Health & Family Welfare, Government of India



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National Centre for Disease Control
Directorate General of Health Services
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Government of India

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Government of India
Ministry of Health & Family Welfare
Directorate General of Health Services



Message

Zoonotic diseases pose a significant and ongoing threat to biodiversity, human and public health and economic stability. Given India's diverse ecosystem, high population density and increasing human-animal action, the country remains particularly susceptible to zoonotic outbreaks. Past occurrences of plague, Nipah virus, avian influenza, leptospirosis, and Kyasanur Forest Disease emphasizes a critical need for robust surveillance, early detection, and a coordinated, intersectoral response.

Recognizing the importance of addressing zoonotic diseases as a public health priority, the Centre for One Health, under the National Centre for Disease Control (NCDC), is committed for strengthening surveillance, prevention, and response mechanisms through an integrated One Health approach. As part of this effort, the Centre for One Health has developed comprehensive learning guides on Priority Zoonotic Diseases. These modules provide scientifically validated, evidence-based guidance on the epidemiology, diagnosis, treatment, and prevention of key zoonotic diseases.

Strengthening disease surveillance, ensuring early diagnosis, and implementing timely interventions through a coordinated and multi-sectoral approach are critical to mitigating the risks associated with zoonotic diseases.

I am pleased to release these learning guides as a significant step in our ongoing efforts to combat zoonotic diseases. I urge all healthcare professionals, veterinary experts, and community leaders to actively utilize these resources to enhance their clinical index of suspicion for zoonosis and eventually contribute towards strengthening our health care system for responding timely and more efficiently to future outbreaks.

(Atul Goel)



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MESSAGE

It is my pleasure to know that the Centre for One Health at NCDC has prepared **Comprehensive Learning Guides** on 10 priority zoonotic diseases, which is now being shared with all concerned.

These resources become relevant in considering; India has encountered multiple zoonotic disease outbreaks in recent years, highlighting their persistent and evolving threat to public health. The re-emergence of **Nipah virus** in Kerala, **Kyasanur Forest Disease (KFD)** in Karnataka, and **avian influenza** across several states underscores the complex interconnections between human, animal, and environmental health. The rising cases of **leptospirosis** and **scrub typhus**, driven by climatic changes and ecological disruptions, further illustrate the unpredictable nature of zoonotic spillovers. With the additional challenges posed by **emerging coronaviruses** and **antimicrobial resistance (AMR)**, there is an urgent need for a **cohesive, evidence-based approach** to mitigate these risks effectively.

In recognition of this pressing need, the **National Centre for Disease Control (NCDC)** has intensified its efforts to fortify **zoonotic disease surveillance, outbreak preparedness, and intersectoral coordination**. As part of this initiative, the **Centre for One Health, NCDC**, has meticulously curated **Comprehensive Learning Guides** on priority zoonotic diseases. These guides, underpinned by the latest scientific insights and global best practices, serve as indispensable resources for **healthcare professionals, veterinarians, and epidemiologists**, equipping them with the knowledge to anticipate, detect, and mitigate zoonotic threats with precision.

To effectively curtail the impact of zoonotic diseases, a **harmonized One Health approach** is paramount—one that transcends disciplinary boundaries and fosters synergies across the human, animal, and environmental health sectors. I urge all stakeholders to **leverage these scholarly resources, enhance disease intelligence networks, and champion research-driven interventions**. By embracing a proactive and interdisciplinary ethos, we can **bolster pandemic preparedness, fortify national health security, and avert future spillover events**.

I sincerely **appreciate** my colleagues at the Centre for One Health, NCDC for their dedication to strengthening zoonotic disease control. Their efforts in enhancing surveillance, preparedness, and public health response are crucial in preventing and managing emerging health threats.

I am glad to see their commitment translating into evidence-based interventions and capacity-building initiatives that enhance zoonotic disease surveillance, outbreak preparedness, and overall health system resilience.

Through continuous innovation, collaboration, and unwavering dedication, we can fortify our health systems and safeguard our nation against zoonotic threats.

Wishing you all happy learning and skill enhancement wherever possible.

रंजन दास 19/3/25
Prof. (Dr.) Ranjan Das



Antibiotic resistance Containment Stewardship: Our Role, Our Responsibility
Judicious Use of Antibiotic: Key to Contain Antibiotic Resistance





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Message

Zoonotic diseases of public health importance continue to pose significant challenges to public health. These diseases, often driven by intricate human-animal-environment interactions, present substantial health risks, particularly to vulnerable populations, and demand a coordinated and robust response from the health sector.

In recognition of the pivotal role healthcare providers play in addressing these challenges, the **Centre for One Health** has developed a **Comprehensive Learning Guide for Priority Zoonotic Diseases**. This guide is designed to build capacity across the health sector, equipping professionals with the essential knowledge and skills needed to identify, manage, and prevent these diseases effectively. By enhancing surveillance systems, fostering rapid and accurate diagnostics, and implementing targeted prevention and control measures, healthcare providers can play a transformative role in mitigating the impact of zoonotic diseases.

The guide emphasizes the importance of building a resilient health workforce capable of responding to outbreaks, engaging communities, and collaborating across disciplines. Through these efforts, we aim to reduce the burden of zoonotic diseases, improve health outcomes, and contribute to the creation of a safer and healthier future.

I encourage all healthcare professionals to utilize this guide as a resource for improving their understanding and response to zoonotic diseases. Together, let us strengthen our health systems and work towards a future free from the threats posed by zoonotic diseases.

Dr. Simmi Tiwari
Joint Director and Head
Centre for One Health



Antibiotic Resistance Containment Stewardship: Our Role, Our Responsibility
Judicious Use of Antibiotic: Key to Contain Antibiotic Resistance



Acknowledgement

The **Comprehensive Learning Guide** for Zika virus has been developed to address the significant public health challenges posed by zoonotic diseases, which have serious health, economic, and social implications, particularly for vulnerable populations. The priority zoonotic diseases necessitate robust surveillance, timely diagnosis, and effective prevention and control measures. This guide aims to strengthen the capacity of healthcare professionals by equipping them with the requisite knowledge and skills to mitigate these public health threats and improve health outcomes.

We acknowledge with deep appreciation the valuable contributions of the **subject matter experts** from leading medical institutions and research organizations, whose guidance and technical expertise has been critical in shaping this **Comprehensive Learning Guide**.

The Centre for One Health, NCDC, expresses its sincere gratitude to Ms. Punya Salila Srivastava, Secretary, Health and Family Welfare, Government of India; Ms. L.S. Changsan, Additional Secretary, Ministry of Health and Family Welfare, Government of India; Prof. (Dr.) Atul Goel, Director General of Health Services (DGHS), Government of India; Ms. Latha Ganapathy, Joint Secretary, Department of Health & Family Welfare, Government of India for their leadership, guidance, and support.

Lastly, we acknowledge the USAID-funded RISE project team, implemented by Jhpiego, for their technical support in drafting and finalizing this guide. Their collaboration has been pivotal in ensuring the quality and relevance of this resource.

Dr. Simmi Tiwari
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Preface

Zika virus disease is a rapidly emerging viral infection caused by the Zika virus. Initially identified in Uganda in 1947, the disease gained significant global attention in 2015, prompting the World Health Organization to declare it a Public Health Emergency of International Concern (PHEIC) on February 1, 2016. This designation was based on the observed temporal link between Zika virus infection and serious health complications such as Microcephaly (a condition characterized by an unusually small head in newborns) and other neurological syndromes, including Guillain Barre Syndrome.

This training module aims to equip learners with the knowledge and tools necessary to educate and train individuals involved in various aspects of Zika case management. It provides a comprehensive overview of the disease, covering its epidemiology, clinical presentation, laboratory diagnosis, treatment availability, and preventive measures. The module is structured in a user-friendly manner, allowing learners to effectively communicate complex scientific concepts to their audience. It contains detailed explanations to enhance understanding and engagement. The content is supported by the latest scientific research and public health guidelines, ensuring the information presented is accurate and up to date.

It is our sincere hope that this module will serve as a valuable resource for learners and educators, enabling them to grasp essential knowledge and skills. By equipping professionals and individuals at risk with the necessary information, we can enhance our collective ability to detect, prevent, and respond to Zika outbreaks, thereby safeguarding public health.

We would like to express our gratitude to all the experts, researchers, and contributors who have provided their invaluable insights and expertise in developing this scientific module. Their dedication and commitment to public health have been instrumental in creating a comprehensive resource that will contribute to the effective management of Zika cases.

We hope you find this module informative, engaging, and empowering. Together, let us work towards building a safer and healthier future by combating Zika Virus disease through knowledge and preparedness.

Abbreviations

AFP	Acute Flaccid Paralysis
AFRO	African Regional Office (WHO)
ALT	Alanine Aminotransferase
AMRO	American Regional Office (WHO)
APHO	Airport Health Organization
AST	Aspartate Aminotransferase
BI	Breteau Index
BSL	Biosafety Level
CD ALERT	Communicable Diseases Alert
CDC	Center for Disease Control and Prevention
CHC	Community Health Centre
CI	Container Index
CMO	Chief Medical Officer
CMV	Cytomegalovirus
CZS	Congenital Zika Syndrome
CSF	Cerebrospinal Fluid
DEIT	District Epidemic Investigation Team
DGHS	Directorate General of Health Services
DNA	Deoxyribonucleic Acid
DSO	District Surveillance Officer
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
EURO	European Regional Office (WHO)
FASD	Fetal Alcohol Spectrum Disorders
FIR	First Information Report
FTA-ABS	Fluorescent Treponemal Antibody Absorption Test
GBS	Guillain-Barré Syndrome
HI	House Index
ICMR	Indian Council of Medical Research
IDSP	Integrated Disease Surveillance Programme
IEC	Information, Education, and Communication
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IVM	Integrated Vector Management
JE	Japanese Encephalitis
LIMS	Laboratory Information Management System
LMP	Last Menstrual Period
MO	Medical Officer
MRI	Magnetic Resonance Imaging
NAAT	Nucleic Acid Amplification Test
NCDC	National Centre for Disease Control
NCVBDC	National Centre for Vector-Borne Disease Control

NHM	National Health Mission
NIV	National Institute of Virology
NPSP	National Polio Surveillance Project
NS1	Non-structural Protein 1
NS5	Non-structural Protein 5
NSAID	Nonsteroidal Anti-Inflammatory Drug
OLE	Oil of lemon eucalyptus
PAHO	Pan American Health Organization
PCR	Polymerase Chain Reaction
PHC	Primary Health Centre
PHO	Port Health Organization
PI	Pupae Index
PMD	Para-Menthane-Diol
PPE	Personal Protective Equipment
PRNT	Plaque Reduction Neutralization Test
RBSK	Rashtriya Bal Swasthya Karyakram
RCVRDL	Regional Coordinating Virology Research and Diagnostic Laboratory
RDT	Rapid Diagnostic Test
RNA	Ribonucleic Acid
RMRC	Regional Medical Research Centre
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SEARO	South-East Asia Regional Office (WHO)
SOP	Standard Operating Procedure
SSO	State Surveillance Officer
TPPA	Treponema Pallidum Particle Agglutination Test
ULV	Ultra-Low Volume
VBD	Vector-Borne Diseases
VDRL	Venereal Disease Research Laboratory Test
WHO	World Health Organization
WPRO	Western Pacific Regional Office (WHO)
ZIKV	Zika Virus
ZVD	Zika Virus Disease

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Introduction to Learning Guide for Zika Virus Disease

Historically, zoonotic diseases have had a significant impact on human evolution, particularly on cultures and societies that domesticated and raised animals for food and clothing. Zoonoses pose a frequent and feared risk to humanity, transcending natural boundaries and occurring worldwide. Their profound effect on the global economy and health is well recognized, ranging from the international movement of animals and the importation of diseases to bans on animal product imports and restrictions on other international trade practices. Thus, zoonoses are no longer confined to national borders, and global surveillance is necessary for effective control.

Controlling zoonoses remains a priority for international agencies, addressing the specific health and economic challenges of each region. They equally impact animal, human, and environmental sectors, causing substantial damage to the nation's economy. To protect all the sectors, it is essential to rapidly identify, diagnose, and manage these diseases. The One Health approach plays a crucial role in this endeavor, as we have learned valuable lessons from the COVID-19 pandemic regarding the need for strong surveillance and diagnostic preparedness to handle future pandemic situations.

To provide comprehensive training, the Center for One Health, NCDC has developed Guide. The guide serves as both trainers' guide and self-directed learning resources. The training focuses on zoonoses and zoonotic diseases of public health importance in India (ZPHI) and emphasizes the One Health approach to responding to zoonotic diseases. By the end of the training, participants should have a solid understanding of the core principles of Zika Virus Disease, its outbreak potential, health emergencies, risk communication, diagnostic procedures, laboratory safety, surveillance and their applications.

The guide is based on national and international guidelines and serve as a comprehensive training resource for individuals involved in zoonotic disease diagnosis and surveillance activities. The curriculum is designed to enable facilitators and learners to undergo step-by-step learning within a specified time frame, ensuring a thorough understanding of each module. The guide caters to various audiences, including clinical care providers, laboratory technicians, and data managers, who are expected to acquire knowledge and competencies beyond their regular field of work.

The Facilitators' Guide (FG) provides instructional processes and supports educators in facilitating and assessing learning. Each module follows a standardized structure, including a session outline, detailed session plans, slide decks, and a wide range of references and resources for further reading. These resources offer comprehensive information on the respective topics.

It helps them understand, identify, and differentiate between zoonotic diseases and equips them with the necessary knowledge and orientation for diagnostics procedures and surveillance systems.

COURSE DESCRIPTION

Course Design

This comprehensive Zika Virus Disease training guide is tailored for medical professionals including doctors, paramedical staff, and laboratory personnel. The course is designed to enhance their understanding and expertise in dealing with Zika Virus Disease cases, spanning various aspects of prevention, diagnosis, and treatment. The training approach emphasizes practical application, aiming to equip participants with hands-on skills and competencies that can be directly applied in their professional roles.

Evaluation Approach

The evaluation approach for the Zika Virus Disease preparedness and response learning guide includes a comprehensive pre- and post-training quiz questionnaire designed to assess participant's knowledge and learning progression. The questionnaire utilizes multiple-choice questions (MCQs) to gauge participant's understanding of Zika Virus Disease before and after the training. This approach allows a clear measurement of knowledge enhancement and provides valuable insights into the effectiveness of the training program.

- ***Pre-Training Quiz:***

Before the training begins, participants will undergo a pre-training questionnaire. This questionnaire serves as a baseline assessment of participant's existing knowledge and awareness of Zika Virus Disease. The pre-training quiz will consist of MCQs that cover various aspects of Zika Virus Disease, including its etiology, modes of transmission, clinical manifestations, preventive measures, and response strategies. The purpose of the pre-training quiz is to establish a starting point and identify areas where participants may require more focused training.

- ***Post-Training Quiz:***

Upon completion of the Zika Virus Disease preparedness and response learning guide participants will be given a post-training quiz questionnaire. This questionnaire will contain a similar set of MCQs as the pre-training quiz, covering the same topics related to Zika Virus Disease. The aim of the post-training quiz is to evaluate participant's knowledge advancement and to measure the effectiveness of the training in enhancing their understanding of Zika Virus Disease.

COURSE SYLLABUS

Course Goals

- Foster an in-depth comprehension of Zika Virus Disease and its diverse forms.
- Equip participants with skills for Zika Virus Disease diagnosis, management, and prevention.
- Emphasize the importance of the One Health approach in addressing zoonotic diseases.
- Develop proficiency in risk associated with the disease, laboratory safety, and surveillance strategies.

Participant's Learning Objectives

By the course's conclusion, participants will be able to:

- Identify various Zika Virus Disease forms and their clinical characteristics.
- Apply diagnostic and treatment protocols for Zika Virus Disease management.
- Implement preventive strategies and outbreak management strategies.

Participant Selection Criteria

Participants for the Zika Virus Disease response and preparedness training should be professionals from various fields involved in emergency response and public health:

- Emergency Responders: Including public health experts, medical personnel, and first responders.
- Laboratory and Biosafety Staff: Those experienced in handling biohazards.
- Government and Decision-Makers: Officials with authority in emergency coordination.
- Community Leaders and Experts: Community influencers, researchers, and mental health specialists.

Using the Resource Guide

All training activities in this course will be conducted in an interactive, participatory manner using the learning guide appropriately as suggested in the course outline in the facilitator's guide. To accomplish this, the trainer will have to change roles throughout the course. For example, the trainer is a facilitator when conducting small group discussions and finally, when objectively assessing performance, the trainer serves as an evaluator.

Composition of participants

The training is intended for 15-20 participants hailing from various state and healthcare fraternities. There can be a single facilitator as an expert of the disease.

COURSE SCHEDULE

Morning Session

Introduce and welcome participants

- Discuss participant expectations and prior knowledge
- Outline the objectives of the course and learning outcomes

Review the course schedule and its components

- Provide an overview of the learning guide
- Conduct a pre-course knowledge assessment to gauge participants' baseline understanding
- Distribute course materials, including handouts and resources

Interactive presentations

- Take Sessions 1-2 and discuss in detail about basics of Zika Virus Disease, its epidemiology and its significant features in humans and animals.
- Interactive discussion and Q&A session after the lecture

Afternoon Session

Interactive presentations

- Take Sessions 3-4 and discuss in detail about diagnosis of Zika Virus Disease, its treatment, prevention & control, and its surveillance & outbreak management.

Debriefing and group discussions on simulation outcomes

- Interactive discussion and Q&A session after the lecture

Review, Assessment, and Closing

- Comprehensive review of key concepts covered during the training
- Interactive quiz to reinforce learning
- Final assessment to measure participants' understanding of course material
- Reflection session: Participants share insights and takeaways from the course
- Closing remarks, thanking participants for their engagement

COURSE OUTLINE

The course outline presented here follows a model structure for the training curriculum. It delineates the enabling objectives necessary to achieve the participant learning goals outlined in the course syllabus. The outline offers recommendations for suitable learning activities and essential resources and materials for each enabling objective. Depending on specific participant requirements, the trainer has the option to introduce supplementary practice activities, devise case studies, role-play scenarios, or tailor learning situations accordingly.

The course outline comprises four columns:

- **Duration:** This column specifies the estimated time allocated for each learning activity. This allocation ensures an equitable distribution of time across the diverse training components.
- **Learning Objectives:** This column lays out the enabling objectives and their alignment with corresponding learning activities. The objectives are sequenced to provide guidance on the training structure. The interplay of objectives and activities—ranging from icebreakers and interactive group tasks to practical exercises and breaks—constitutes the overall training progression.
- **Pre-requisite Knowledge/Skill:** This column addresses the foundational knowledge or skills that participants should possess prior to the session. It underscores the importance of existing competencies for effective engagement in the training.
- **Competencies to be Achieved:** The fourth column enumerates the competencies participants are expected to acquire by the conclusion of the session. It underscores the practical outcomes that participants will attain through engagement in the training.

AGENDA

S. No	Topic	Duration
1.	Welcome & Introduction of the training agenda and setting ground rules	15 mins
2.	Ice Breaker: Talk about yourself • Contribution to the management & control of zoonoses • Expectations from the training	30 mins
3.	Training objectives	15 mins
4.	Pre-Test Quiz	20 mins
5.	Session 1: Introduction to Zika Virus	30 mins
6.	Session 2: Vector and Vector Management	30 mins
7.	Session 3: Diagnosis and Surveillance	30 mins
8.	Session 4: Prevention & Treatment	30 mins
13.	Post - Test Quiz	20 mins
14.	Q/A session, review, and discussion	15 mins
15.	Closing remarks	05 mins

Session-1

Introduction to Zika Virus

Course No. 001	Session 1: Introduction to Zika Virus
Duration	30 minutes
Learning Objective	• Describe disease overview • Describe the history and epidemiology • Describe the disease transmission and clinical manifestations • Describe Zika in Animals
Pre-requisite knowledge/skill	• Basic understanding of viral infections and vector-borne diseases. • Familiarity with general concepts of public health and disease surveillance. • Awareness of mosquito-borne illness transmission and prevention strategies.
Competencies to be achieved	• Learners would gain knowledge of the basic characteristics and features of Zika Virus Disease • Understanding of the characteristics and causative agent, transmission, and clinical symptoms associated with the disease. • Describing the transmission cycle and factors associated with the disease. • Understand the disease patterns in Animals.

INTRODUCTION TO ZIKA VIRUS DISEASE

Zika virus is a mosquito-borne flavivirus primarily transmitted by the *Aedes* species of mosquitoes, particularly *Aedes aegypti*, which also spreads dengue, chikungunya, and yellow fever. The virus was first discovered in 1947 in monkeys in the Zika Forest of Uganda, with the first human case reported in 1952. Since then, Zika has caused sporadic outbreaks in tropical regions, including Africa, Southeast Asia, the Pacific Islands, and more recently, the Americas.

Zika virus infections are often asymptomatic or present mild symptoms, including fever, rash, joint pain, headache, and conjunctivitis (red eyes). These symptoms typically last for 2 to 7 days. However, the virus gained global attention due to its link with severe neurological complications, especially when contracted during pregnancy. Congenital Zika syndrome can result in microcephaly, a condition where babies are born with unusually small heads and underdeveloped brains, along with other developmental disorders.

Zika is mainly transmitted through mosquito bites, but it can also spread through sexual contact, blood transfusions, and from pregnant women to their unborn babies. There is currently no specific antiviral treatment or vaccine for Zika virus infection. Management of the disease involves supportive care, such as rest, hydration, and pain relief.

Prevention efforts focus on mosquito control measures, such as removing standing water where mosquitoes breed, using insect repellents, wearing protective clothing, and implementing screens or nets. Public health campaigns also emphasize the importance of avoiding travel to Zika-endemic regions, especially for pregnant women, to reduce the risk of infection.

In the past decade, Zika virus infection has been linked to an increased occurrence of Guillain-Barré syndrome. The virus gained significant attention when a large epidemic occurred in Brazil in 2015, and a connection between Zika virus infection and microcephaly (abnormally small head size) was first noted. Similar findings were observed in French Polynesia upon retrospective review. Between February and November 2016, the World Health Organization (WHO) declared a Public Health Emergency of International Concern (PHEIC) due to the observed association between microcephaly, other neurological disorders, and Zika virus. The causal link between Zika virus and congenital malformations was subsequently confirmed.

Zika virus disease cases globally decreased from 2017 onwards, but the virus transmission persists at low levels in several countries in the Americas and other endemic regions. Notably, in 2019, the first instances of locally transmitted mosquito-borne Zika virus disease were reported in Europe, and in 2021, Zika virus outbreak activity was detected in India. To date, evidence of mosquito-transmitted Zika virus infection has been reported in 89 countries and territories. However, global surveillance for Zika virus remains limited.

It was initially identified in Uganda in 1947 and has since caused outbreaks in Africa, the Americas, Asia, and the Pacific. Notably, significant outbreaks occurred in French Polynesia in 2013 and Brazil in 2015. Starting in 2015, Zika virus rapidly expanded its geographic range, with mosquito-borne transmission reported in 67 countries. Recognizing the severity of the situation, the World Health Organization declared Zika virus disease a Public Health Emergency of International Concern (PHEIC) on February 1, 2016.

On July 8, 2021, the first case of Zika virus infection in Kerala state, southwest India, was confirmed through laboratory testing. The Zika virus is identified as a single-stranded RNA virus belonging to the Flavivirus genus within the Flaviviridae family. Nonhuman and human primates serve as reservoirs for the virus.

Common symptoms of Zika virus infection include skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. These symptoms are typically mild and last for 2-7 days. Zika virus is primarily transmitted through anthroponotic (human-to-vector-to-human) transmission, with reported cases of perinatal, in utero, and potential sexual and transfusion transmission events.

HISTORY AND GLOBAL EPIDEMIOLOGY

Zika virus was first identified in Uganda in 1947 during a routine surveillance study for yellow fever. Initially discovered in a rhesus monkey, the virus was later found in mosquitoes, confirming its mosquito-borne transmission. By 1952, the first human cases of Zika were reported in Uganda and Tanzania. For the next few decades, from the 1960s to the 1980s, sporadic human cases were observed in Africa and Asia, but these were generally mild and without major outbreaks.

In 2007, Zika made a significant leap when it caused its first large outbreak on the Pacific Island of Yap, marking a shift in its behavior. Further outbreaks occurred in French Polynesia in 2013, where retrospective analysis revealed a possible link between Zika infection and neurological complications, including Guillain-Barré syndrome and congenital abnormalities like microcephaly.

In 2015, a major epidemic in Brazil brought Zika to global attention, particularly after a surge in microcephaly cases among newborns. This prompted Brazil to declare a national public health emergency. By February 2016, the World Health Organization (WHO) declared Zika a Public Health Emergency of International Concern (PHEIC), recognizing the association between the virus, congenital malformations, and neurological disorders.

From 2017 onwards, global Zika cases declined, although transmission persisted in several countries, including new instances of locally transmitted cases in Europe in 2019 and an outbreak in India in 2021. To date, Zika virus transmission has been reported in 89 countries and territories, although global surveillance remains limited.

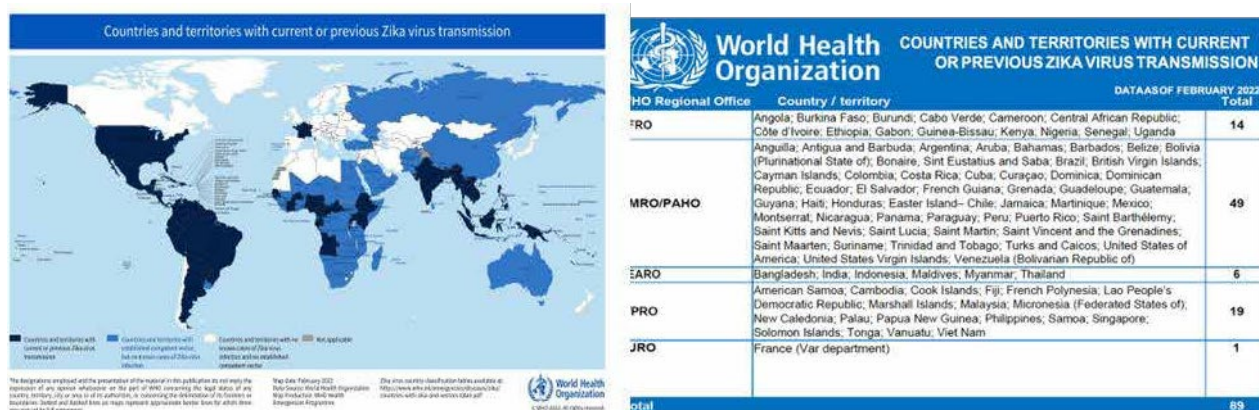


Fig 1: Countries and territories with current or previous Zika virus transmission (WHO)

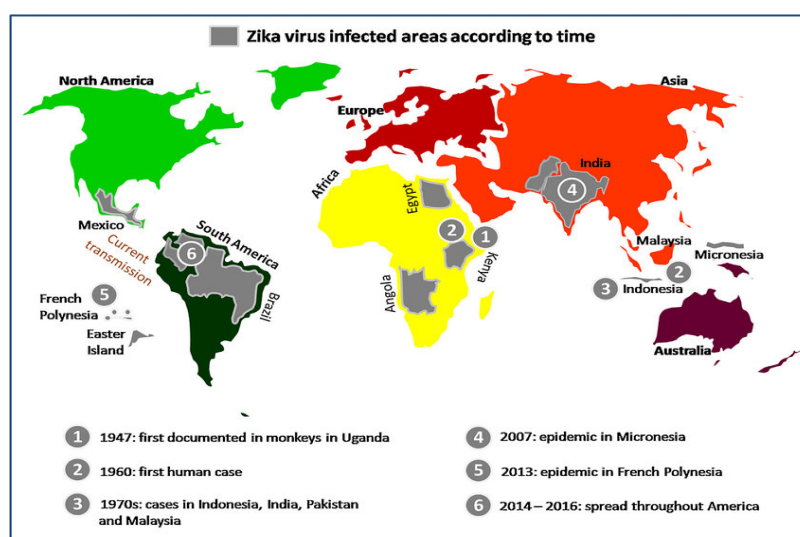


Fig 2: Zika virus infection timeline

(Image Source: N. Rashad, A. Tasnia Zika virus: Epidemiological study and its association with public health risk Journal of Infection and Public Health)

TRANSMISSION AND CLINICAL MANIFESTATIONS

Zika virus is primarily transmitted through the bite of infected *Aedes* mosquitoes, especially *Aedes aegypti* and *Aedes albopictus*. However, other modes of transmission include mother-to-child transmission during pregnancy, which can lead to congenital complications like microcephaly. Zika can also spread through sexual contact, making it unique among mosquito-borne viruses. Blood transfusions and organ transplantation from infected individuals have also been identified as a potential source of transmission. These various transmission routes highlight the complexity of controlling Zika virus outbreaks and the importance of preventive measures.

PROTECT YOUR FAMILY AND COMMUNITY

HOW ZIKA SPREADS

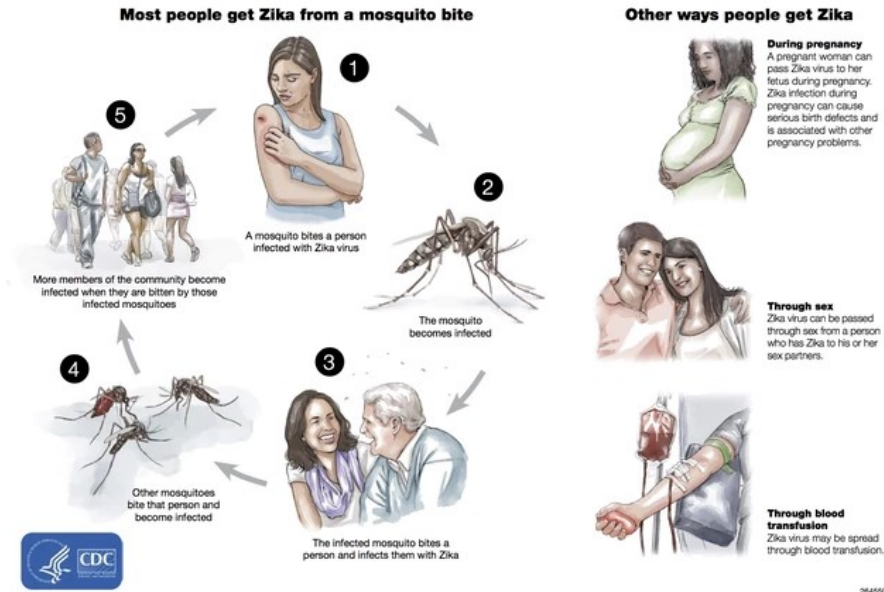


Fig 3: Zika Virus Spread (CDC)

Mosquito Bite

These mosquitoes breed in standing water, such as buckets or flowerpots, and are active both indoors and outdoors, biting people during the day and sunset. When a mosquito bites a person infected with Zika virus during the first week of their infection, it can become infected and later spread the virus to other individuals through its bites. This mosquito-borne transmission plays a crucial role in the spread of Zika, especially in tropical and subtropical regions.

Mother to Child

An additional concern related to Zika is its potential transmission from a pregnant woman to her fetus. In cases where a pregnant woman is infected with the Zika virus, there is a heightened risk of certain birth defects in the developing fetus. Thus, it becomes crucial for pregnant women to take extra precautions to minimize the risk of exposure to mosquitoes carrying the virus. The transmission from mother to child in all trimesters resulting in abnormalities in fetus. The abortion rate is high in early trimester.

Sexual Transmission

Zika can be transmitted through sexual activity from an infected person to their sex partners. To mitigate this risk, the use of condoms is recommended during sexual encounters. Both male and female condoms serve as protective barriers, reducing the likelihood of acquiring Zika through sexual transmission.

Blood Transfusion

The presence of Zika virus in an infected person's blood typically lasts for about a week. If you reside in or have recently traveled to an area where there is a risk of Zika transmission and you develop symptoms, it is advisable to consult your doctor or healthcare provider. They may conduct blood or urine tests to confirm the presence of Zika. Following a Zika infection, individuals are likely to develop immunity that provides protection against future infections.

CLINICAL MANIFESTATION

80% people are symptomatic. Zika virus disease infections often go unnoticed as many individuals won't display symptoms or will experience only ones. The most common manifestations of Zika include exanthema/rash, arthralgia, conjunctivitis, myalgia, headache and diarrhoea. Generally, Zika is a mild illness, and its symptoms persist for several days to a week. Most people do not require hospitalization, and fatalities due to Zika are extremely rare. Consequently, individuals infected with Zika may not be aware of their condition. Notably, the symptoms of Zika closely resemble those of other viruses transmitted through mosquito bites, such as dengue and chikungunya and thus a high degree of clinical suspicion and establishing an epidemiological link (such as contact with a confirmed case of ZVD, history of residing in or travelling to a local area with known Zika virus transmission 2 weeks prior to the onset of symptoms) is important.

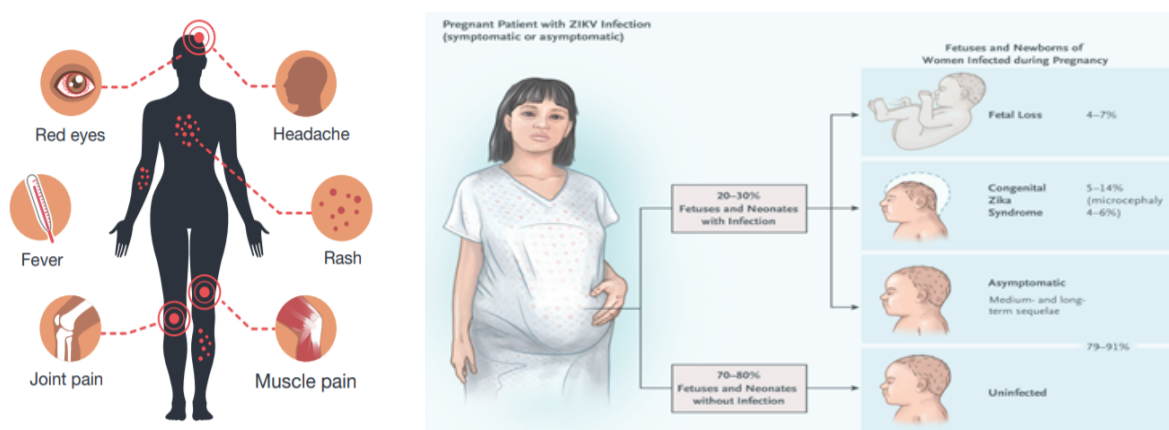


Fig 4: Clinical Manifestations of Zika virus infection (CDC)

ZIKA VIRUS INFECTION IN ANIMALS

Zika can infect nonhuman primates, such as apes and monkeys, as evidenced by its initial discovery in a monkey with mild symptoms in the Zika Forest of Uganda during the 1940s. The frequency of Zika infections in apes and monkeys remains unclear, and existing studies suggest that when infected, they may exhibit mild symptoms or a fever. Further research is essential to comprehensively understand the extent to which apes and monkeys contribute to the transmission of Zika to humans. Limited research indicates that apes and monkeys infected with Zika during pregnancy may experience severe fetal brain defects, emphasizing the potential impact on offspring.

In the late 1970s, a study conducted in Indonesia revealed that horses, cows, water buffaloes (carabaos), goats, ducks, and bats could be infected with Zika. However, there is no evidence to suggest that these animals show signs of illness or have the capacity to transmit Zika to humans. Notably, there have been no reported cases of pets or other animal types falling ill due to Zika.

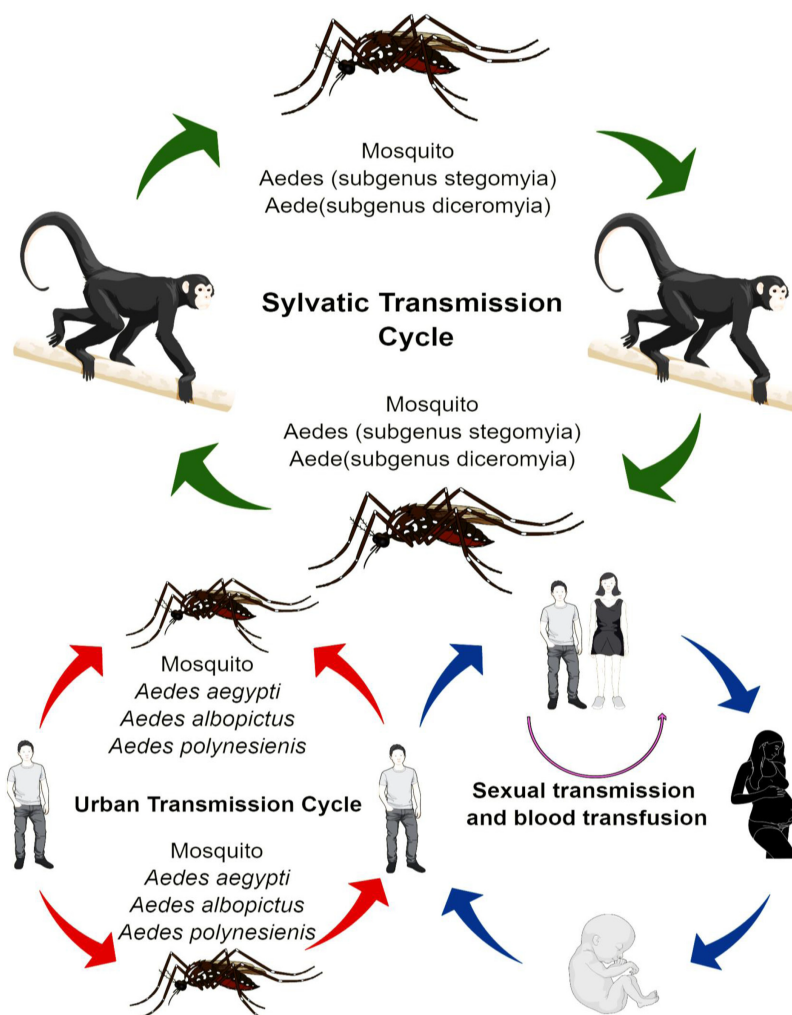


Fig 5: Zika Virus Disease Overview in animals

(Image Source: Zika Virus: An Emerging Worldwide Threat)

- Zika virus disease is an emerging viral disease transmitted through the bite of an infected Aedes mosquito (*Ae. aegypti* and *Ae. albopictus*).
- First identified in Uganda in 1947. Outbreaks of this disease have been recorded in Africa, the Americas, Asia and the Pacific. Large outbreaks in French Polynesia and Brazil in 2013 and 2015 respectively.
- From 2015 onwards, the geographical range of Zika virus has expanded rapidly with mosquito-borne transmission of the virus reported in 67 countries.
- World Health Organization has declared Zika virus disease to be a Public Health Emergency of International Concern (PHEIC) on 1st February 2016.
- On 8 July 2021, Zika virus (ZIKV) infection was laboratory-confirmed in a resident of Kerala state, south-west India. This represents the first Zika virus disease case ever reported from Kerala.



Source: Yale News File view (http://www.yale.edu/newsroom).

Computer-Aided Design, Inc.

ZIKA VIRUS DISEASE

- **Agent:** Zika virus is a single-stranded RNA virus
- **Genus:** *Flavivirus*
- **Family:** *Flaviviridae*.
- **Reservoir:** Nonhuman and human primates.
- **Symptoms:** Skin rashes, Conjunctivitis, Muscle and joint pain, Malaise, and Headache.
- **Incubation:** These symptoms are usually mild and last for 2-7 days.
- **Transmission:** Anthroponotic (human-to-vector-to-human) transmission. Perinatal, in utero, and possible sexual and transfusion transmission events have also been reported.



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ZIKA VIRUS DISEASE

1947	First identified in monkeys in Uganda
1952	The first human case identified in Uganda and the United Republic of Tanzania
1969–1983	Spread of Zika to Asian countries like India, Indonesia, Malaysia and Pakistan and sporadically to Africa.
2007	Zika reaches the Pacific island Yap from Asia and Africa to cause first ever large outbreak in humans.
2013–2014	Outbreaks in four other Pacific islands: French Polynesia, Easter Island, the Cook Islands, and New Caledonia. The French Polynesia outbreak, generating thousands of suspected infections.
2015	7000 cases of skin rash reported in northeastern states of Brazil. All mild cases with no reported deaths
2016	High incidence of Zika in the regions of America.
2020	22,885 cases of ZIKV disease in the American region. 18,941 cases (14% laboratory confirmed) reported in Brazil.

WHO declared the association of Zika infection with microcephaly and other neurological disorders which constitutes a Public Health Emergency of International Concern in 2016

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ZIKA VIRUS DISEASE

World map showing the global distribution of HIV/AIDS cases. The map is color-coded: dark blue for high prevalence, medium blue for moderate prevalence, and light blue for low prevalence. High prevalence is concentrated in sub-Saharan Africa and parts of Asia. Moderate prevalence is seen in Latin America and the Caribbean, and parts of Europe and Asia. Low prevalence is seen in North America, Australia, and parts of Europe and Asia. A legend at the bottom left explains the color coding and provides additional statistics.



Source - N. Bishof, A. Tomin. The virus: Epidemiological study and its association with public health risk. *Journal of Infection and Public Health*

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ZIKA VIRUS DISEASE

[illegible]

Countries and territories with current or previous Zika virus transmission

World Health Organization		
COUNTRIES AND TERRITORIES WITH CURRENT OR PREVIOUS ZIKA VIRUS TRANSMISSION		
WHO Regional Office	Country / Territory	DATE AS OF FEBRUARY 2016
AFRO	Angola, Burkina Faso, Burundi, Cabo Verde, Cameroon, Central African Republic, Côte d'Ivoire, Ethiopia, Gabon, Guinea-Bissau, Kenya, Nigeria, Senegal, Uganda, Anguilla, Antigua and Barbuda, Argentina, Aruba, Bahamas, Barbados, Belize, Bolivia (Plurinational State of), Botswana, Saint Eustatius and Saba, Brazil, British Virgin Islands, Cayman Islands, Colombia, Costa Rica, Cuba, Curaçao, Dominica, Dominican Republic, Ecuador, El Salvador, French Guiana, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Ecuador, Chile, Jamaica, Martinique, Mexico, Montserrat, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, Saint Barthélemy, Saint Kitts and Nevis, Saint Lucia, Saint Martin, Saint Vincent and the Grenadines, Saint Maarten, Suriname, Trinidad and Tobago, Turks and Caicos, United States of America, United States Virgin Islands, Venezuela (Bolivarian Republic of)	14
AMRO/PANO	Bangladesh, India, Indonesia, Maldives, Myanmar, Thailand	49
SEARO	American Samoa, Cambodia, Cook Islands, Fiji, French Polynesia, Lao People's Democratic Republic, Marshall Islands, Malaysia, Micronesia (Federated States of), New Caledonia, Palau, Papua New Guinea, Philippines, Samoa, Singapore, Solomon Islands, Tonga, Vanuatu, Viet Nam	6
WPRO	France (Var department)	19
EURO		1
Total		90

Centre for One Health, NCDC, MoHFW

ZIKA VIRUS DISEASE

Factors responsible for transmission

1. Mosquito bites
2. Mother to Child
3. Sexual Contact
4. Blood transfusion
5. Organ Transplant



Source: Asia Pacific Region, Communications and Technical

Centre for One Health, NCDC, MoHFW

ZIKA VIRUS DISEASE

Through mosquito bites



- Zika virus primarily spreads to humans through the bites of infected Aedes mosquitoes, specifically Ae. aegypti and Ae. albopictus, which also transmit dengue and chikungunya.
- These mosquitoes lay eggs in or near standing water, like buckets and flowerpots, and are active both indoors and outdoors. They bite people during the day and night.
- The virus is transmitted to mosquitoes when they bite an infected person within the first week of their infection. Subsequently, these infected mosquitoes can transmit the virus to other individuals through bites.

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ZIKA VIRUS DISEASE

In Pregnant Women



Zika virus can be transmitted from a pregnant woman to her fetus, potentially leading to microcephaly and other severe brain defects.

Transmission can occur during pregnancy or around the time of birth.

While the virus has been detected in breast milk, confirmed cases of transmission through breastfeeding are not yet established.

However, it is advised to mothers, even those infected or in Zika-prone areas, to continue breastfeeding, as current evidence suggests the benefits outweigh the risk.

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ZIKA VIRUS DISEASE

Notes

- Zika can be transmitted through sexual contact, even when the infected person is asymptomatic.
- The virus can be passed before, during, and after the onset of symptoms, and there's a possibility of transmission by individuals who never show symptoms.
- Ongoing studies are investigating the duration of Zika presence in semen and vaginal fluids, as it tends to persist longer in semen compared to other bodily fluids like vaginal fluids, urine, and blood.
- Protective measures during sexual activity are recommended to reduce the risk of transmission.



ZIKA VIRUS DISEASE

- Transmission may happen from blood and blood products. However, one case of ZVD transmission is reported through platelet transfusion in Brazil
- Certain human cell and tissue-based products, such as umbilical cord blood, gestational tissues, and reproductive tissues, can harbor Zika virus even months after the initial infection.

- If a patient has been diagnosed with Zika virus in the last 4 months, inform the blood center before donating. It may take up to 4 months for some individuals to clear the virus from their blood. If a patient is diagnosed with Zika shortly after donating blood, notify the blood center. Proven Zika virus cases (laboratory confirmed) should not donate blood in 4 months.
- Write proven Zika virus cases (laboratory confirmed) should not donate blood in 4 months.
- If a patient diagnosed with or potentially exposed to Zika, refrain from donating cells or tissues such as umbilical cord blood, placenta, reproductive tissues (eggs or semen), organs or other gestational tissues.



ZIKA VIRUS DISEASE

Host Susceptibility & High-Risk Groups:

- **Primary Host:** Humans
- **Vectors:** Aedes mosquitoes (Ae. aegypti and Ae. albopictus)
- **High-Risk Groups:** Pregnant women, infants, and individuals with weakened immune systems.

- **Viral Entry:** Zika virus enters the body through mosquito bites or sexual transmission.
- **Viral Replication:** The virus replicates in various cell types, including skin cells, dendritic cells, and endothelial cells.
- **Immune Response:** The body's immune response is often mild, but it can contribute to tissue damage.
- **Neurotropism:** In some cases, the virus can reach the central nervous system, leading to neurological complications.



- People infected with the Zika virus usually do not experience any symptoms or experience very mild symptoms
- The mild symptoms, when they do occur, are similar to those of dengue and chikungunya, develop within 3 to 12 days and last for about a week.

CDC and international partners have concluded that Zika virus infection during pregnancy is a cause of **microcephaly** and other severe brain defects.

- brain abnormalities.
- eye defects.
- hearing loss.
- limb defects.

Current CDC research suggests that **GUILLAIN-BARRÉ SYNDROME** is strongly associated with Zika



ZIKA VIRUS DISEASE

[illegible]

Most Zika virus infections are asymptomatic, with no noticeable symptoms.

Symptoms, when present, usually appear 3–14 days after infection. The typical duration of symptoms is 2–7 days.

Common mild symptoms include rash, fever, conjunctivitis, muscle and joint pain, malaise, and headache.

Laboratory confirmation is necessary for the accurate diagnosis of Zika virus infection.

Similar symptoms are shared with other arboviral and non-arboviral diseases.

<https://www.cdc.gov/zika/faq/index.html>
<https://www.merckmanuals.com/home/infectious-diseases/zika-virus/zika-virus-overview>

Centre for One Health, NCDC, MoHFW

ZIKA VIRUS DISEASE

Notes

Zika Virus Disease Overview in animals

Reservoir- Human and nonhuman primates.

Agent-Zika virus is a single-stranded RNA virus

Transmission- Zika spreads to people primarily by the bite of an infected mosquito.

Symptom- Unlike humans, wild mammals with ZIKV infection display few clinical signs such as transient rash, conjunctivitis, and fever.

Incubation period- ZIKV RNA in the blood plasma is brief, lasting approximately 7–10 days and peaking 2–6 days after subcutaneous infection.

Treatment-There is no specific treatment available for Zika virus infection or disease

The CDC reports there is no evidence that the Zika virus is spread to people from contact with animals.



Source: Zika Virus: An Emerging Worldwide Threat

Centre for One Health, NCDC, MoHFW

ZIKA VIRUS DISEASE

Session-2

Diagnosis and Surveillance

Course No. 002	Session 2: Diagnosis and Surveillance
Duration	30 minutes
Learning Objective	• Describe the diagnosis, case definition, diagnostic algorithm, and methodologies • Describe disease surveillance
Pre-requisite knowledge/skill	• Understanding of clinical diagnostic processes and case definitions in public health. • Familiarity with laboratory testing methods • Basic knowledge of epidemiological surveillance systems and data reporting.
Competencies to be achieved	• Understanding disease diagnosis, and methodologies • Understanding disease surveillance

DIAGNOSIS

Zika virus infection often presents a diagnostic challenge due to its frequent lack of symptoms. A comprehensive approach is recommended, combining clinical evaluation with laboratory testing. During the initial consultation, healthcare providers will inquire about symptoms and travel history to areas with known Zika transmission, as outlined by the World Health Organization (WHO). Laboratory confirmation then relies on either detecting the Zika virus's genetic material using nucleic acid amplification tests (NAATs) like RT-PCR in blood or urine samples, particularly within the first few days of symptoms. Alternatively, serological tests like ELISA can identify antibodies against Zika, indicating a past infection. However, these may cross-react with other flaviviruses, requiring additional confirmatory tests for definitive diagnosis, as highlighted by the European Centre for Disease Prevention and Control (ECDC). This combined approach ensures accurate diagnosis and informs appropriate management, especially for pregnant women or those with severe symptoms.

The steps followed to diagnose Zika Virus disease infection include;

1. Clinical evaluation
2. Laboratory diagnosis
3. Differential diagnosis
4. Consideration for Special Populations
5. Follow-up and additional testing

Clinical Evaluation: While laboratory testing plays a crucial role in diagnosing Zika virus infection, the initial step often involves a thorough clinical evaluation by a healthcare provider. This evaluation aims to gather information that increases the suspicion of Zika and helps guide further diagnostic steps. Here's a breakdown of what a clinical evaluation for Zika typically entails:

- a. **Symptom Review:** The doctor and healthcare providers will inquire about the presence, nature, and duration of any symptoms you might be experiencing. Common Zika symptoms include fever, rash, joint pain, conjunctivitis (red eyes), and headache, but most infected individuals (around 80%) are asymptomatic.
- b. **Travel History:** Information regarding recent travel to areas with known Zika transmission, as outlined by the World Health Organization (WHO), is crucial. Even if symptoms are mild or absent, the history of travel to a Zika-affected region significantly increases the suspicion of Zika infection. (link for real time Zika countries-https://cdn.who.int/media/docs/default-source/documents/emergencies/zika/countries-with-zika-and-vectors-table_21-may-2024.pdf?sfvrsn=b37d66a_1)
- c. **Pregnancy Status:** Pregnant women who have travelled to areas with Zika virus transmission should mention about their travel during ante-natal visits in order to be assessed and monitored appropriately.
- d. **Past Medical History:** The doctor may inquire about any past illnesses, particularly other flavivirus infections like dengue or chikungunya, as they can share similar symptoms. Understanding your overall health can help rule out other potential causes for your symptoms.

Limitations of Clinical Evaluation: Due to the often-asymptomatic nature of Zika and the overlap of symptoms with other infections, clinical evaluation alone cannot definitively diagnose Zika. However, it provides valuable information that helps guide further testing and risk assessment.

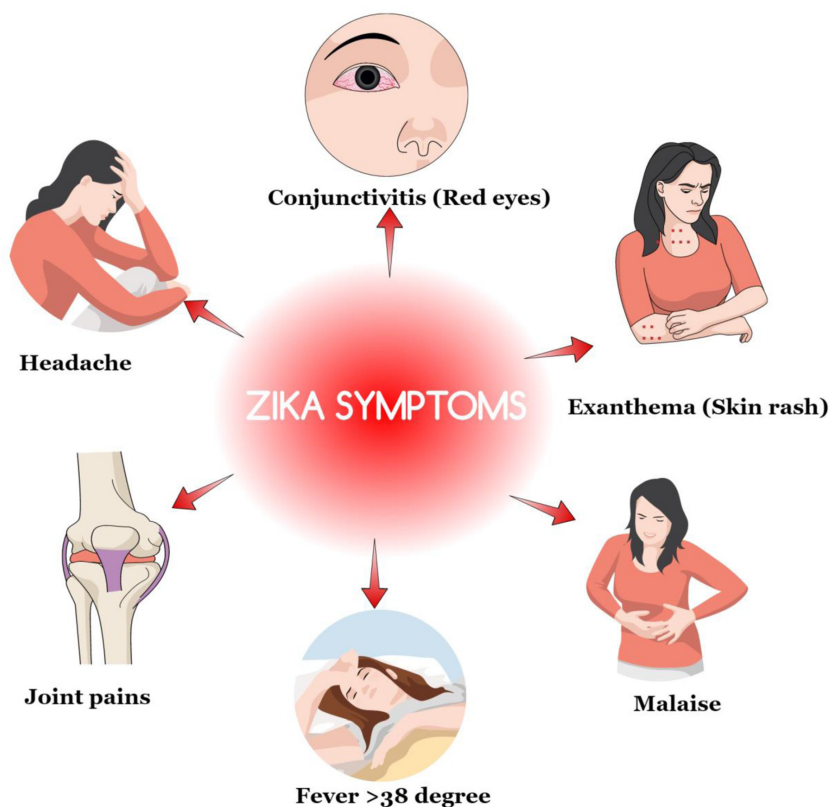


Fig 6: Symptoms of Zika virus infection

(Image source: https://www.frontiersin.org/files/Articles/248771/fmicb-08-00581-HTML/image_m/fmicb-08-00581-g001.jpg)

COMPLICATIONS OF ZVD IN HUMANS

Zika virus infection during pregnancy, particularly in the first and second trimesters, poses significant risks to fetal development. While many infected mothers may experience mild or no symptoms, the virus can cross the placental barrier and infect the developing fetus, leading to severe complications. The most notable outcome is **Congenital Zika Syndrome (CZS)**, characterized by microcephaly, craniofacial dysmorphism, neuromotor abnormalities, seizures, arthrogryposis, ocular abnormalities, deafness, and neurofacial abnormalities. Structural brain abnormalities, such as calcifications, enlarged ventricles, and malformations of the cerebellum and corpus callosum, are hallmarks of CZS, caused by the destruction of neural progenitor cells and inflammation in the womb. The risk of congenital malformations, estimated at 5–15% in infants born to infected mothers, is highest during the first trimester and can occur following both symptomatic and asymptomatic infections. Zika infection in pregnancy can also result in fetal loss, stillbirth, and preterm birth. In addition to congenital complications, Zika virus has been linked to **Guillain-Barré syndrome**, neuropathy, and myelitis, particularly in adults and older children. Differential diagnosis of CZS is challenging due to symptom overlap with other

congenital infections and conditions. Despite its typically mild presentation in adults, the potential for severe congenital outcomes underscores the critical importance of preventive measures and early diagnosis in managing Zika virus infection.

Some of the conditions to consider during differential diagnosis include:

- Cytomegalovirus (CMV) infection
- Toxoplasmosis
- Rubella infection
- Syphilis
- Fetal alcohol syndrome
- Other genetic or chromosomal disorders

Clinicians should consider the mother's medical history, prenatal testing results, and the infant's physical examination findings to differentiate between congenital Zika syndrome and other conditions.

DIFFERENTIAL DIAGNOSIS

The clinical manifestations of Zika Virus Disease (ZVD) closely resemble those of several other diseases, presenting challenges in accurate diagnosis. Diseases characterized by skin rash, including Rubella, Measles, Chikungunya, Scarlet Fever, Meningococcal Infection, Parvovirus, Human Immunodeficiency Virus primary infection, Ehrlichiosis, and Lyme disease, share symptomatic similarities with ZVD. Diarrheal diseases such as Rotavirus and other enteric infections, as well as conditions with neurological manifestations like Meningoencephalitis of other etiologies, diphtheria, enterovirus, and neuromyelitis optica (Devic's Syndrome), can manifest similarly to ZVD. Hemorrhagic fevers, including Leptospirosis, Yellow Fever, and Severe Dengue, exhibit overlapping symptoms with ZVD. Lastly, blood dyscrasias such as proliferative hematological disorders, alterations in coagulation, and paraproteinemia, also present challenges in distinguishing from ZVD due to shared clinical features. The identification and differentiation of ZVD from these similar conditions require careful consideration of the specific clinical context and may necessitate diagnostic testing for accurate and targeted management.

Table 1: Differential Diagnosis for Zika Virus Disease and Related Conditions

Disease	Epidemiological Clue	Key Clinical Features	Key Distinguishing Features	Laboratory Tests and Results
Zika Virus	Recent travel to Zika-endemic areas; mosquito exposure	Fever, rash (maculopapular), conjunctivitis, arthralgia	Rash (maculopapular) , conjunctivitis, arthralgia; no bleeding, mild symptoms , no neurological involvement	RT-PCR: Positive, NS1 antigen (+) ; Mild AST/ALT elevation
Dengue Fever	Recent travel to endemic areas, mosquito exposure	Fever, retro-orbital pain, rash, bleeding (milder)	Severe retro-orbital pain , prominent bleeding (nose/gums); often tourniquet test positive	NS1 antigen: Positive, IgM/IgG ELISA: Positive; Mild liver enzyme elevation
Chikungunya	Recent travel to endemic areas, mosquito exposure	High fever, joint pain, rash (maculopapular), severe arthralgia	Severe joint pain (often debilitating); rash similar to Zika but more pronounced arthralgia	RT-PCR: Positive, NS1 antigen (+) , IgM/IgG ELISA
Japanese Encephalitis	Exposure to Culex mosquitoes in rural/agricultural regions	Fever, headache, altered mental state, seizures	Progressive neurological symptoms with fever; endemic in rural areas; often severe encephalitis	CSF analysis: Lymphocytic pleocytosis, increased protein; JE-specific IgM antibody detection in CSF
Malaria	Recent travel to endemic areas; exposure to Anopheles mosquitoes	Fever, chills, sweating, splenomegaly, headache	Cyclic fever (classic), splenomegaly ; malaria parasites visible in blood smear	Blood smear: Malarial parasites present; Rapid Diagnostic Test (RDT): Positive
Zika Virus (in Pregnancy)	Travel to Zika-endemic areas during pregnancy	Fever, rash, conjunctivitis, arthralgia	Congenital Zika Syndrome (CZS) in infants: microcephaly, neurological and structural brain abnormalities	RT-PCR: Positive, NS1 antigen (+) , IgM/IgG ELISA

Key Differences Highlighted:

- **Zika Virus:** Rash with **conjunctivitis and arthralgia**, no bleeding or severe systemic involvement.
- **Dengue Fever:** Prominent **retro-orbital pain** and bleeding (nose/gums).
- **Chikungunya:** Severe **debilitating joint pain** with rash.
- **Japanese Encephalitis:** Severe **neurological involvement** (e.g., altered mental state, seizures).
- **Malaria:** **Cyclic fever**, chills, and **splenomegaly** with **blood smear-confirmed parasites**.
- **Zika (in Pregnancy):** Risk of **Congenital Zika Syndrome**, with microcephaly and brain abnormalities in infants.

Table 2: Differential Diagnosis for Congenital Zika Syndrome and Related Conditions

Disease	Epidemiological Clue	Key Characteristics	Laboratory Tests	Expected Results
Congenital Zika Syndrome (CZS)	Recent travel to or residence in Zika-affected areas	Microcephaly , intracranial calcifications, ocular abnormalities, hearing loss, neurological deficits	Serology: IgM and IgG antibodies against Zika virus; PCR: Detection of Zika virus RNA in amniotic fluid, cord blood, or tissue	Positive Zika virus-specific IgM and/or IgG antibodies; Positive Zika virus RNA detection
Congenital Cytomegalovirus (CMV) Infection	No specific epidemiological clue	Hearing loss , intellectual disability, vision impairment, seizures, neurological abnormalities	Serology: IgM and IgG antibodies against CMV; PCR: Detection of CMV DNA in urine, saliva, or cerebrospinal fluid	Positive CMV-specific IgM and/or IgG antibodies; Positive CMV DNA detection
Congenital Toxoplasmosis	Exposure to infected cats or undercooked meat	Chorioretinitis , hydrocephalus, intracranial calcifications, neurological impairment	Serology: IgM and IgG antibodies against <i>Toxoplasma gondii</i> ; PCR: Detection of <i>T. gondii</i> DNA in amniotic fluid, cord blood	Positive <i>T. gondii</i> -specific IgM and/or IgG antibodies; Positive <i>T. gondii</i> DNA detection
Congenital Rubella Syndrome	Recent maternal rubella infection	Cataracts , deafness, heart defects, intellectual disability	Serology: IgM and IgG antibodies against rubella virus	Positive rubella-specific IgM and/or IgG antibodies
Fetal Alcohol Spectrum Disorders (FASD)	Maternal syphilis infection	Facial abnormalities , growth deficits, neurodevelopmental disorders	No specific laboratory tests; diagnosis based on clinical findings and maternal history	Clinical findings consistent with FASD
Genetic or Chromosomal Disorders	Family history, genetic testing	Various physical and intellectual disabilities	Karyotype analysis, chromosomal microarray analysis, and specific gene mutation testing	Abnormal karyotype or genetic mutations

Key Differences Highlighted:

- **Congenital Zika Syndrome** is characterized by **microcephaly** and brain abnormalities, unique to Zika infection.
- **Congenital CMV** often presents with **hearing loss** and vision impairment without microcephaly.
- **Congenital Toxoplasmosis** involves **chorioretinitis** and hydrocephalus.
- **Congenital Rubella Syndrome** is distinguished by **cataracts** and cardiac defects.
- **Congenital Syphilis** includes a **rash** and hepatosplenomegaly.
- **FASD** is associated with **maternal alcohol use** and lacks specific laboratory markers.
- **Genetic/Chromosomal Disorders** require **genetic testing** for confirmation.

Table 3: Clinical Overlap of Zika Virus, Chikungunya, and Dengue

Disease	Dengue	CHIK	Zika
Fever	++++	+++	+++
Myalgia	+++	++++	++
Arthralgia	+++	++++	++
Periarticular Oedema	–	–	++
Maculopapular exanthema	–	++	+++
Retro-orbital pain	++	+	++
Conjunctivitis	–	+	+++
Hepatomegaly	–	+++	–
Bleeding	+	–	–

(Source: <https://www.elsevier.es/en-revista-revista-medica-del-hospital-general-325-articulo-clinical-differential-diagnosis-dengue-chikungunya-S0185106316301135>)

Table 4: Comparison of Zika virus infection with other prevalent diseases in India with focus on differential diagnosis

Disease	Zika	Malaria	Dengue Fever	Chikungunya	Japanese Encephalitis	Japanese B Encephalitis
Vector	Aedes mosquitoes (Aedes aegypti, Aedes albopictus)	Anopheles mosquitoes	Aedes mosquitoes	Aedes mosquitoes	Culex mosquitoes	Close contact
Symptoms	Fever, rash, joint pain, conjunctivitis	Fever, chills, sweating, headache	High fever, severe headache, joint pain	Fever, joint pain, rash, headache	Fever, headache, convulsions	Rash, fever, swollen lymph nodes
Pregnancy	Congenital birth defects (microcephaly) possible	Not a major concern in pregnancy	Not a major concern in pregnancy	Not a major concern in pregnancy	Not a major concern in pregnancy	Congenital defects
Diagnosis	RT-PCR for viral RNA, serology (IgM/IgG)	Blood smear	NS1 antigen, IgM/IgG	Serology, RT-PCR	Serology, RT-PCR	Serology, RT-PCR

Consideration for Pregnant and Newborn

Test for serology within 7 days and followed by RT PCR. If negative, then repeat. For pregnancy-Anti Natal screening for fetal abnormalities, mother has infection at time of delivery then newborn has to be screened for diseases and new born abnormalities. Due to the severe risks Zika poses to

Similarly, newborns born to mothers with positive or inconclusive Zika tests, or those exhibiting microcephaly or other birth defects, should be tested for Zika to determine if congenital Zika syndrome is a possibility. This targeted testing approach ensures timely diagnosis and allows for proper management of both the mother and infant.

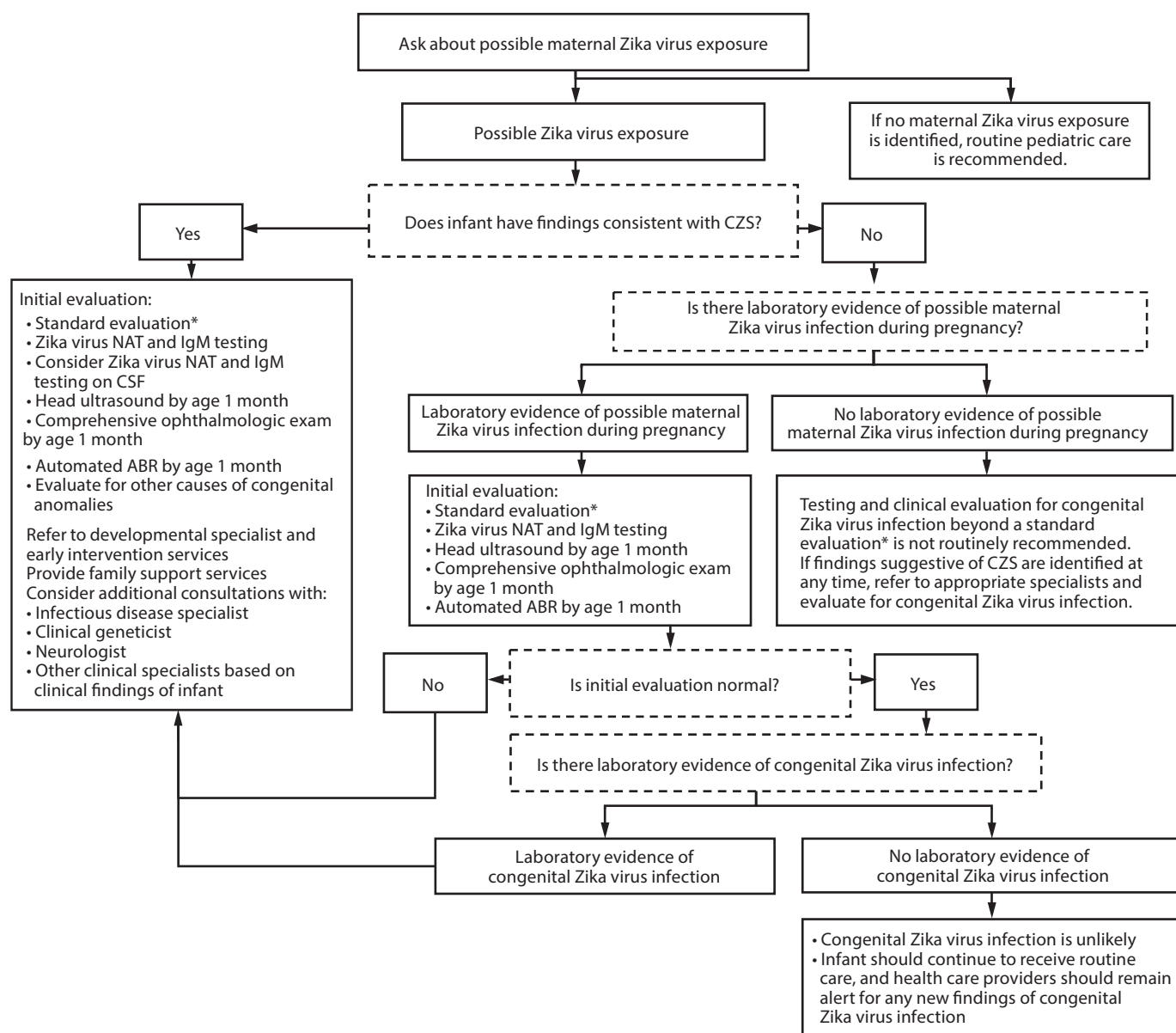


Fig 7: Recommendations for evaluation of infants with possible congenital Zika virus infection based on clinical findings, maternal testing, and infant testing results

(Source: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5689094/>)

Follow-up and additional testing

While initial tests might be negative, follow-up testing may be necessary if Zika infection remains a concern. This emphasizes the importance of a comprehensive approach. Additionally, for pregnant women or newborns with suspected Zika, imaging tests like ultrasounds or MRIs may be recommended to detect abnormalities potentially caused by the virus. Ultimately, accurate Zika diagnosis is crucial. It not only guides patient management but also informs public health measures to prevent further spread of the virus.

LABORATORY DIAGNOSIS

A BSL-2 laboratory is suitable for working with Zika virus. This level of containment is designed to protect laboratory workers and the environment from exposure to moderate-risk agents.

During the first 5 days after the onset of symptoms for diagnosis by RT PCR and after 5 days onset of symptoms for diagnosis by serology (ELISA).

Overview of the Zika virus investigation/diagnosis process

Several methods can be used for diagnosis, such as viral nucleic acid detection, virus isolation and serological testing. Diagnosis by serology can be difficult as the virus can cross-react with other flaviviruses. Thus, viral nucleic acid detection remains the preferred method for diagnosis.

Clinical Specimens and Storage of Specimen

Parameter	Details
Type of Sample	Serum
Volume of Sample	About 500 µl or available quantity of serum. Higher volume is preferred.
Storage (0-48 hours)	Refrigerate at 2-8 °C
Storage (48 hours - 7 days)	Freeze at -10 to -20 °C
Storage (>7 days)	Freeze at -70 °C for long-term preservation

Guidelines for specimen Collection

- A BSL-2 containment level is required to handle suspected samples.
- Consider all specimens as potentially hazardous/ infectious.
- Handle all specimens with gloves in a secure manner.
- Place each specimen into a separate container labeled with the patient's name and identification number, the collection site, the date of collection and the time of the collection.
- Do not contaminate the outside of the specimen container.
- Do not handle laboratory requisition forms with gloves.

Sample Collection

Collecting body fluids other than serum or CSF (e.g., urine, amniotic fluid)

- Provide 0.5–1.0 mL of the specimen in a sterile screw-capped vial secured with a small piece of thermoplastic, self-sealing lab film.
- Please ensure a tight seal as leaking specimens cannot be accepted.
- For RNA NAT testing, specimens should be kept cold (2–8°C) or frozen ($\leq -20^{\circ}\text{C}$) for storage and shipping.
- For virus isolation testing, specimens should be frozen as soon as possible ($\leq -20^{\circ}\text{C}$).
- Store the remainder of the sample at $\leq -70^{\circ}\text{C}$ for long-term storage.
- If collecting both urine and semen, obtain the urine specimen first.

Collecting serum or cerebrospinal fluid (CSF)

- At least 1.0 mL* of serum or CSF is required for testing.
- Transfer serum or CSF to a plastic tube measuring approximately 50 mm tall and 15 mm in diameter (e.g., 1.8 mL cryotube or 2.0 mL microtube) with a screw cap.
- For virus isolation testing, specimens should be frozen as soon as possible ($\leq -20^{\circ}\text{C}$).
- For antibody and RNA NAT testing, specimens should be kept cold (2–8°C) or frozen ($\leq -20^{\circ}\text{C}$).
- Store the remainder of the sample at $\leq -70^{\circ}\text{C}$ for long-term storage.

Information to be sent to the Apex laboratories

- a. Every sample should be accompanied by appropriate data form
- b. NOTE: Following details are must in the data form:

Parameter	Details
Date of Onset of Symptoms	Record the exact date when symptoms began.
Date of Specimen Collection	Record the date the sample was collected for diagnosis.
Pertinent Travel History	Note any travel within 3 months prior to symptom onset.
Female Patient Details	Include details of LMP (last menstrual period) and pregnancy status, if any.
Packaging	Ensure proper triple-layered packaging for transport according to biosafety standards.

Guidelines for Transporting Clinical Samples

Parameter	Details
Temperature for Transport	Samples to be sent on ice (maintain +2 to +8 °C).
Sample Labeling and Documentation	Original samples must be packed, labeled, marked, and documented as Category B.
Packaging Requirements	Use standard triple-layered packing as per guidelines for Category B samples.
Prior Notification	Sender must provide prior intimation about sample shipment to RCVRDL.

Note: In case you have any logistic problem or suggestion, kindly contact NIV, Pune

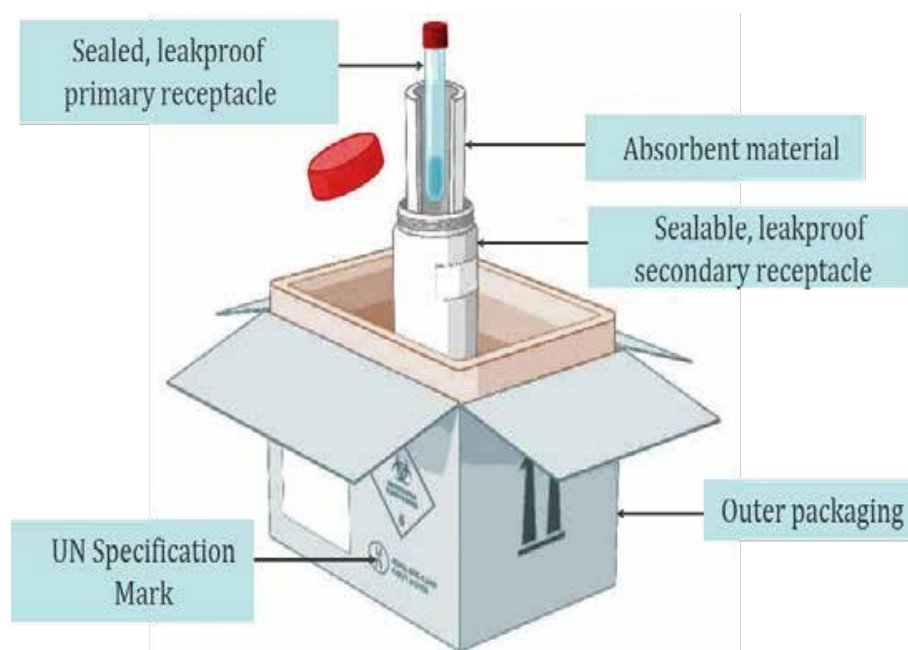


Fig 8: Triple packaging system

Sample registration

The sample registration will be done in LIMS, which is the prerequisite for further processing.

Sample handling

The sample aliquoting and maintenance team should be supervised by senior staff. Each sample should be divided into three aliquots. For samples with limited volume, diagnostic PCR should be prioritized, ensuring a minimum backup of 200 µl for reference purposes. The standard aliquoting format will be as follows:

S.No	Name	Sample sent Form	NIV ID	Aliquot 1	Aliquot 2	Aliquot 3	ELISA 20 ul	PCR 140 ul	Isolation 200 ul

After aliquoting the empty vials as well as gloves should be packed in biohazard bags, secured and loaded to autoclave for decontamination. The note of **“Do not open”** should be mentioned on the autoclave by aliquoting team.

Processing of sample

The clinical samples which are negative for Dengue and Chikungunya should be screened for ZIKV diagnosis.

Zika virus is confirmed through PCR (polymerase chain reaction) and virus isolation from blood samples, though serological diagnosis can be challenging due to cross-reactivity with other flaviviruses. While clinical evaluation offers important clues, a definitive diagnosis requires laboratory testing. These tests aim to either detect the Zika virus directly or assess the body's immune response to the infection. Together, these laboratory methods are crucial for accurate diagnosis and effective management of Zika virus infections.

Diagnostic RT-PCR

- Nucleic acid detection is performed using reverse transcriptase-polymerase chain reaction (RT-PCR) targeting the non-structural protein 5 (NS5) genomic region.
- RT-PCR (standard and quantitative) offers a rapid, specific, and sensitive method for early ZIKV detection.

Sample Types and Detection Timeline

- Viral RNA has been detected in serum up to day 10 after symptom onset.
- ZIKV RNA has also been detected in urine and saliva samples.

Recommended Sampling

- Serum samples are recommended within the first 5 days after symptom onset for optimal results.
- Further studies are required to validate findings in urine or saliva samples.

Sampling Window

Time Frame	Sample Type	Diagnostic Tests
Day 1 - Day 5 after symptom onset	Serum	RT-PCR (Standard & Quantitative) targeting NS5 genomic region
Day 1 - Day 10 after symptom onset	Serum	RT-PCR (Standard & Quantitative) for Zika Virus RNA detection
Day 1 - Day 10 after symptom onset	Urine	RT-PCR (if further validation studies are available for urine samples)
Day 1 - Day 10 after symptom onset	Saliva	RT-PCR (if further validation studies are available for saliva samples)

RT-PCR Standardization

- The RT-PCR test available at NIV is standardized based on published primers.

Detection of IgM antibodies to Zika virus by diagnostic ELISA

Convalescent Phase Testing (> 5 Days):

- Serology:
 - ◇ IgM antibodies are tested in blood.
 - ◇ Not the primary diagnostic method due to high cross-reactivity with other flaviviruses.
- Plaque Reduction Neutralization Test (PRNT):
 - ◇ Used for confirmatory diagnosis.
 - ◇ Highly specific for differentiating ZIKV from other flaviviruses.

Documentation:

Test readings and interpretations are to be maintained in a file with test number and date for easy reference.

Virus Isolation

Viral isolation is not regarded as a diagnostic tool and is recommended only for supplemental research studies in public health surveillance.

Choosing the Right Test

The selection of the most appropriate test depends on several factors, including:

- Symptoms: Presence or absence of symptoms and their timing.
- Time since suspected exposure: NAATs are preferred for recent infection.
- Pregnancy status: Pregnant women with suspected Zika infection may require specific testing strategies.

Limitations of Laboratory Diagnosis

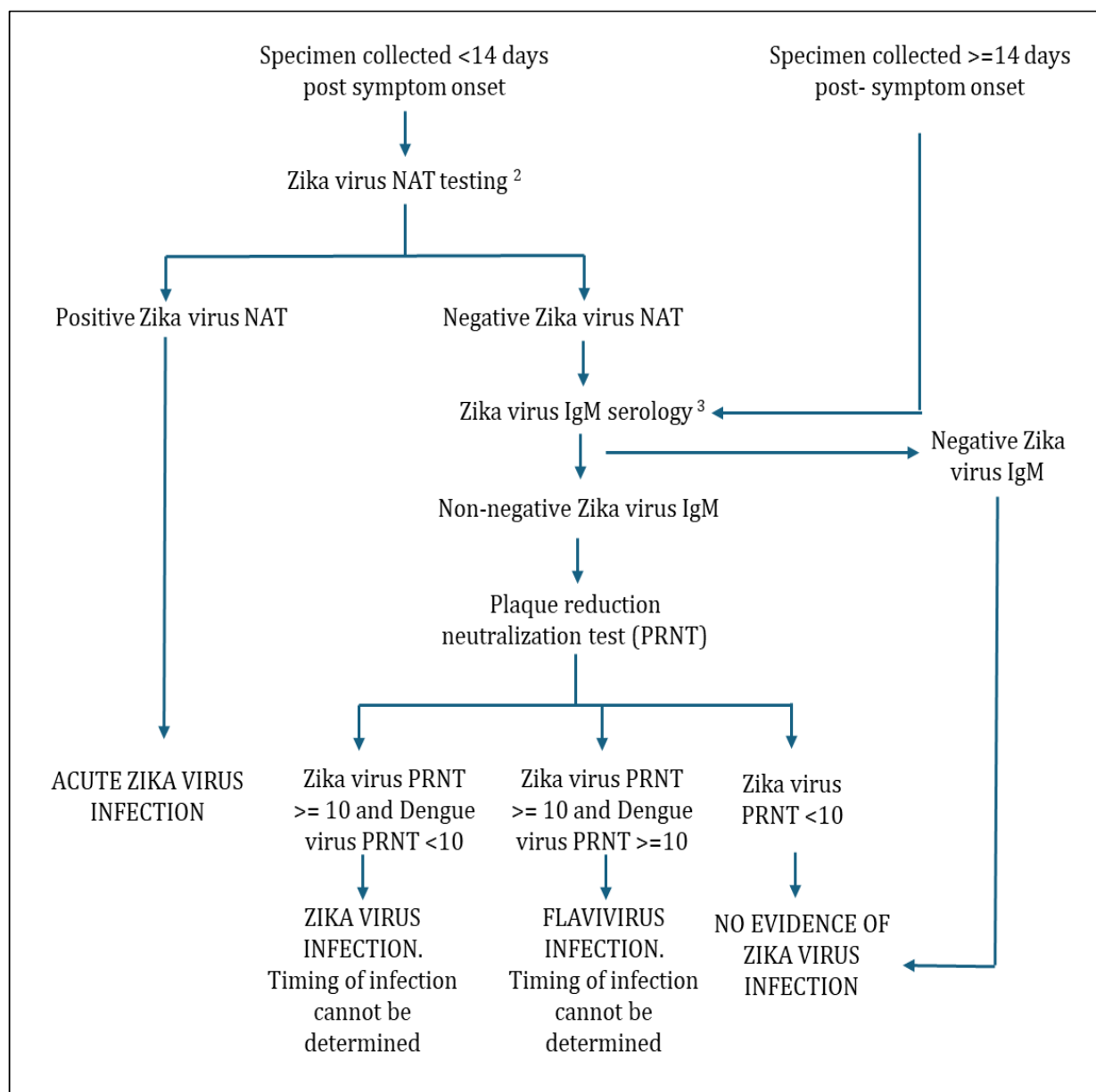
NAATs have a limited window of detection. Serological tests can be positive for other flaviviruses, requiring confirmatory testing.

Early and accurate diagnosis allows for proper management of the infection, especially in high-risk groups like pregnant women. (Refer Ministry of Health & Family Welfare guidelines- <https://www.mohfw.gov.in/?q=media/disease-alerts/national-guidelines-zika-virus-disease/action-plan-managing-zika-virus-disease>)

Testing Recommendations for Symptomatic Non-pregnant Individuals

The World Health Organization (WHO) provides specific testing recommendations for symptomatic non-pregnant individuals with potential exposure to the Zika virus. Viral nucleic

acid detection remains the preferred diagnostic method, with nucleic acid testing (NAT) recommended within 7–14 days of symptom onset. Paired serum and urine serve as primary diagnostic specimens, while cerebrospinal fluid (CSF) may also be used when collected alongside serum. Zika virus RNA can sometimes be detected early in the illness using real-time reverse transcription polymerase chain reaction (r-RT PCR) testing. PCR testing should be performed on serum, urine, or CSF samples collected within two weeks and up to 12 weeks after symptom onset. Given the cross-reactivity with other flaviviruses, confirmatory plaque reduction neutralization tests (PRNT) may be required.



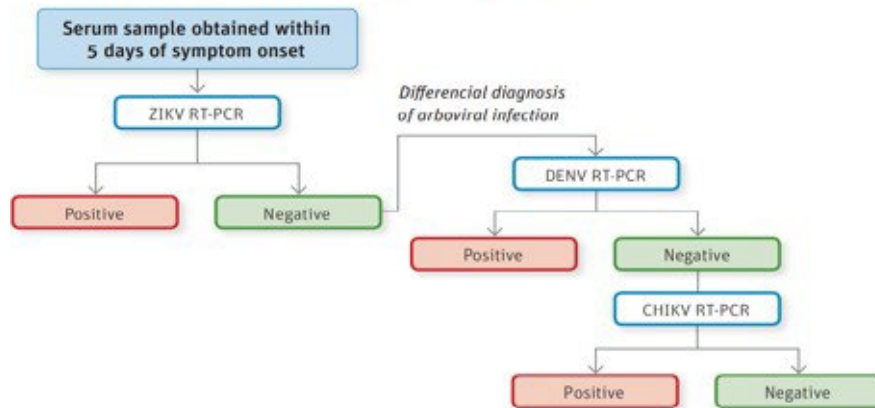
¹NAT testing is not recommended for specimens obtained ≥14 days post-symptom onset.

²Acceptable specimens for NAT testing include serum, or patient-matched serum and urine. Repeat NAT testing of a positive result is not indicated. Dengue and chikungunya virus NAT testing should be performed for patients at risk of exposure and with clinically compatible illness.

³Dengue IgM serology should also be performed for patients at risk of exposure, and with clinically compatible illness.

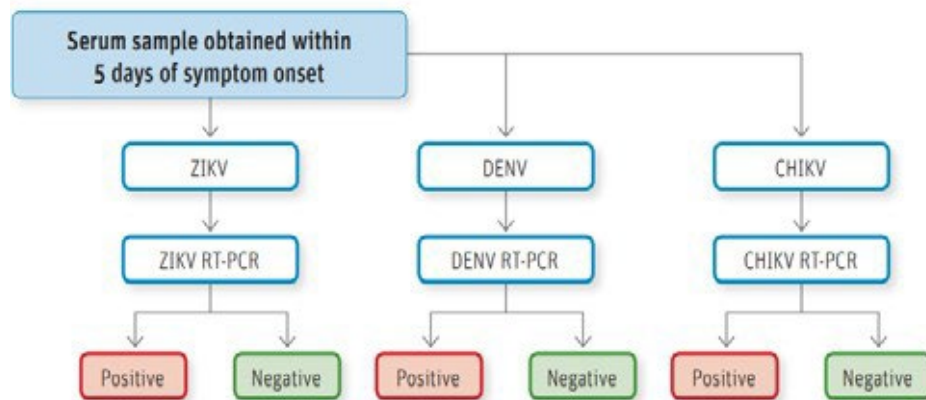
Fig 9: Testing recommendations for symptomatic non-pregnant individuals (WHO)

A. Algorithm for virologic testing using serial monoplex RT-PCR of suspected cases of ZIKV infection in areas where other arboviruses* are in circulation

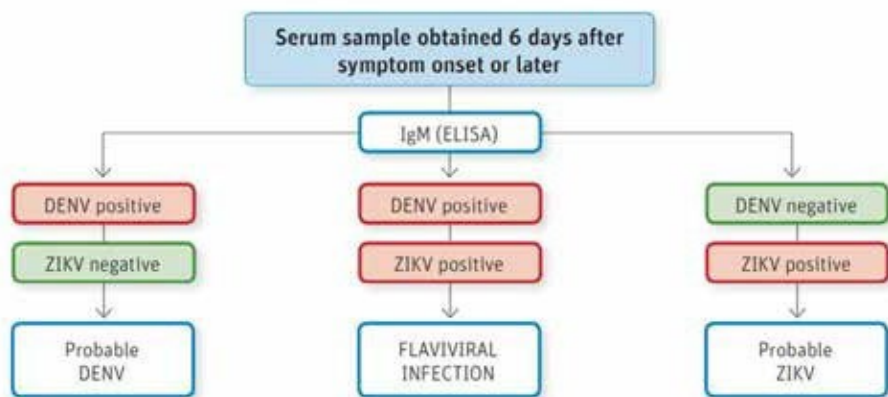


*Based on clinical presentation and the local epidemiology of the area where the case was detected, yellow fever virus should be part of the differential diagnosis.

B. Algorithm for virological testing using a multiplex RT-PCR of suspected cases of ZIKV infection in areas where other arboviruses are in circulation.



C. Algorithm for serological detection in suspected cases of ZIKV infection in areas with circulation of other arboviruses*



* Depending on clinical presentation and local epidemiology in the area where the case has been detected, the inclusion of yellow fever in the differential diagnosis is advised.

Fig 10: Diagnostic Algorithm for Differential Diagnosis by RT-PCR and ELISA (WHO)

List of laboratories for Plaque Reduction Neutralization Tests (PRNT)

For Zika virus diagnosis are performed at highly specialized laboratories that have the necessary biosafety facilities and expertise. Some of the key laboratories equipped for PRNT include:

- 1. National Institute of Virology (NIV), Pune**
 - An apex virology research institute under the Indian Council of Medical Research (ICMR).
 - Performs confirmatory PRNT testing for Zika virus and other arboviruses.
- 2. Regional Medical Research Centres (ICMR RMRCs)**
 - Some RMRCs, especially those focusing on arboviral diseases, may be equipped to perform PRNT under ICMR directives.
- 3. National Centre for Disease Control (NCDC), Delhi**
 - Operates as a central reference lab and may coordinate confirmatory testing or facilitate PRNT through other labs.
- 4. ICMR-designated Virus Research and Diagnostic Laboratories (VRDLs)**
 - Selected VRDLs with advanced facilities may perform PRNT, especially in cases of arboviral outbreaks.

PRNT is a highly specialized and labor-intensive test requiring high biosafety standards. Therefore, confirmatory PRNT is centralized and typically conducted at reference laboratories like NIV, Pune. Local testing centers or hospitals usually send samples to these reference labs for PRNT confirmation.

Nucleic acid amplification tests (NAAT) stand out as the preferred diagnostic approach for detecting viral genomic material, offering a high level of specificity and the ability to confirm the presence of infection. However, it is important to note that despite the reliability of molecular testing, isolated cases of false positive NAAT results have been reported. The occurrence of such false positives may be influenced by factors such as the specific type of NAAT assay used and the characteristics of the patient population being tested, especially in scenarios where Zika virus testing is performed on populations not aligning with recommended testing algorithms.

Given the temporal nature of Zika virus RNA in serum and urine, it's crucial to understand that a negative NAAT result does not necessarily exclude recent Zika infection. To address this limitation and enhance diagnostic accuracy, Zika virus immunoglobulin (IgM) antibody testing is recommended in certain situations. IgM levels typically become detectable within the first week after the onset of symptoms and can persist for up to 12 weeks post-symptom onset or exposure, with the potential for longer persistence in some cases.

Zika virus antibody testing, particularly through IgM, with its own set of challenges, due to cross-reactivity with other flaviviruses. This cross-reactivity increases the likelihood of false-positive results compared to NAAT. To address these complexities, Plaque Reduction Neutralization Tests (PRNT) are employed. PRNTs are quantitative assays that measure virus-specific neutralizing antibody titers, aiding in resolving false-positive IgM results. While many state health departments and commercial laboratories perform dengue and Zika virus NAAT and IgM antibody testing,

confirmatory PRNT is currently available through a limited number of state health departments and the CDC.

It is essential for healthcare professionals and laboratory personnel to be aware of the limitations and intricacies associated with these testing methods. The decision to employ confirmatory PRNT, especially in areas with a high prevalence of dengue and Zika virus neutralizing antibodies, should be based on informed considerations of local prevalence and the observed performance of PRNT to confirm IgM test results. This training emphasizes the importance of a nuanced and comprehensive approach to Zika virus diagnosis, considering the strengths and limitations of each diagnostic method.

SURVEILLANCE

The Integrated Disease Surveillance Programme (IDSP) is set to enhance its monitoring efforts for fever cases across the . In this regard, it is now mandatory for all health services, both in the government and private sectors, to promptly report any suspected cases of Zika virus disease. Rigorous fever surveillance needs to be conducted, with a particular focus on clusters of cases. Samples collected from these clusters will undergo testing to confirm the presence of Zika virus disease through laboratory diagnosis.

Suspected case: Patient with skin rash or elevation of body temperature more than or equal to 37.2 degrees with one or more of the following symptoms (not explained by other medical conditions):

- Arthralgia or myalgia
- Non purulent conjunctivitis or conjunctival hyperemia
- Headache or malaise

Confirmed case: A suspected case with laboratory positive result for the specific detection of ZIKV by RT-PCR.

All pregnant women in the affected State will be under surveillance for febrile illness. In cases of pregnant women with suspected infection, notification must be mandatory and immediate, made within 48 hours. They will be tested for Zika virus, and their fetus monitored for development of microcephaly. There shall be weekly reporting of microcephaly by the RBSK under NHM. Surveillance will continue for acute neurological illnesses. Suspect case of GBS, if any, shall also be tested for Zika virus disease. Monthly data, State-wise, on GBS will be submitted to the Control Room of DGHS by IDSP in co-ordination with NPSP.

To enhance the surveillance and control measures for Zika virus, a comprehensive strategy has been implemented at all International Airports. Travelers passing through these airports will encounter informative billboards and signage addressing Zika virus disease. These displays will instruct individuals returning from countries affected by Zika to report to immigration authorities if they are experiencing symptoms of febrile illness.

In conjunction with these measures, the Airport/Port Health Organization (APHO/PHO) will establish quarantine and isolation facilities at identified airports. This initiative aims to swiftly identify and manage potential cases, preventing the spread of the Zika virus within the country. The Directorate General of Civil Aviation, Ministry of Civil Aviation, is actively involved in this effort. They will issue instructions to all international airlines, urging them to adhere to recommended guidelines for aircraft disinfection. This collaborative approach ensures that aircraft, a critical point of entry, are disinfected in accordance with established health protocols.

To further fortify the preventive measures, APHOs will disseminate guidelines for aircraft disinfection, aligning with the International Health Regulations, to all international airlines. Concurrently, the APHOs will collaborate with the National Center for Vector Borne Diseases Control (NCVBDC) to monitor and implement appropriate vector control measures. This joint effort aims to curb the transmission of Zika virus and safeguard public health by maintaining rigorous standards across the international travel network. Through these proactive measures, the authorities are committed to ensuring the safety and well-being of travelers and preventing the potential introduction and spread of Zika virus within the country.

Diagnosis of Zika Virus

ZIKV can be identified in various clinical samples, including blood (plasma, serum), cerebrospinal fluid (CSF), urine, saliva, breast milk, semen, vaginal secretions, amniotic fluid, and tissues.

Diagnosing Zika virus infection involves a combination of clinical evaluation, laboratory tests, and understanding the patient's exposure history.

Here are the main steps and methods used in the diagnosis of Zika virus infection:

Clinical Evaluation	Laboratory Tests	Differential Diagnosis	Consideration for Special Populations	Follow-up and additional testing
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ZIKA VIRUS DISEASE

Clinical Evaluation

- Symptoms are non-specific and the differential diagnosis represents a challenge. The most common symptom is fever
- pleuritic rash - generalized rash, begins with trunk and affects even palms and soles.
- maculopapular exanthema
- arthralgia
- non-purulent conjunctivitis
- myalgia
- headache
- retro-ocular pain
- Other, rarer symptoms include haematospermia and lower urinary tract symptoms.
- Symptoms manifest in a period of 3-12 days



Exposure History: Evaluating the patient's travel history to areas with active Zika virus transmission, exposure to mosquitoes, or sexual contact with someone who might have been exposed to Zika virus is crucial.

Image source: <https://www.frontiersin.org/articles/10.3389/fmicb.2017.01771/full#fig-01>

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ZIKA VIRUS DISEASE

Congenital Zika Syndrome (CZS)

- A group of birth defects caused by Zika virus infection in the womb.
- These include
 - microcephaly
 - cranio facial dysmorphism
 - neuro motor abnormalities
 - seizures
 - arthrogryposis
 - ocular abnormalities
 - deafness
 - neuro facial abnormalities.



Hallmarks include microcephaly (small head size) and structural brain abnormalities.

- These abnormalities can lead to:
 - Developmental delays
 - Seizures
 - Hearing and vision problems
 - Movement difficulties

CDC source: Zika associated birth defects

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ZIKA VIRUS DISEASE

Risk

- Around 5% of babies born to infected mothers develop CZS.
- There's no specific treatment, but early diagnosis and support can improve outcomes.



Mechanism of Action in Pregnancy:

- **Placental Infection:** Zika virus can cross the placenta and infect the developing fetus.
- **Fetal Damage:** Viral infection can disrupt fetal brain development, leading to microcephaly and other severe birth defects.
- **Immune-Mediated Damage:** The maternal immune response to the virus may also contribute to fetal damage.

<https://www.cdc.gov/zika/nczid/nczid-publications-section-2/frequency-of-zika-associated-birth-defects>

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ZIKA VIRUS DISEASE

Notes

Differential Diagnosis for Zika Virus Disease and Related Conditions:

Disease	Epidemiological Cue	Key Clinical Features	Key Distinguishing Features	Laboratory Tests and Results
Zika Virus	Recent travel to Zika-endemic areas; mosquito exposure	Fever, rash (maculopapular), conjunctivitis, arthralgia	Rash (maculopapular) , conjunctivitis, arthralgia; no bleeding; mild symptoms , no neurological involvement	RT-PCR: Positive, NS1 antigen (+); Mild AST/ALT elevation
Dengue Fever	Recent travel to endemic areas, mosquito exposure	Fever, retro-orbital pain, rash, bleeding (milder)	Severe retro-orbital pain , prominent bleeding (nose/gums); often tourniquet test positive	NS1 antigen: Positive, IgM/IgG ELISA: Positive; Mild liver enzyme elevation
Chikungunya	Recent travel to endemic areas, mosquito exposure	High fever, joint pain, rash (maculopapular), severe arthralgia	Severe joint pain (often debilitating); rash similar to Zika but more pronounced arthralgia	RT-PCR: Positive, NS1 antigen (+); IgM/IgG ELISA
Japanese Encephalitis	Exposure to Culex mosquitoes in rural/agricultural regions	Fever, headache, altered mental state, seizures	Progressive neurological symptoms with fever; endemic in rural areas; often severe encephalitis	CSF analysis: Lymphocytic pleocytosis, increased protein; JE-specific IgM antibody detection in CSF
Malaria	Recent travel to endemic areas; exposure to Anopheles mosquitoes	Fever, chills, sweating, splenomegaly, headache	Cyclic fever (classic), splenomegaly ; malaria parasites visible in blood smear	Blood smear: Malarial parasites present; Rapid Diagnostic Test (RDT) : Positive
Zika Virus (in Pregnancy)	Travel to Zika-endemic areas during pregnancy	Fever, rash, conjunctivitis, arthralgia	Congenital Zika Syndrome (CZS) in infants: microcephaly, neurological and structural brain abnormalities	RT-PCR: Positive, NS1 antigen (+); IgM/IgG ELISA

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ZIKA VIRUS DISEASE

Differential Diagnosis for Congenital Zika Syndrome and Related Conditions

Disease	Epidemiological Cue	Key Characteristics	Laboratory Tests	Expected Results
Congenital Zika Syndrome (CZS)	Recent travel to or residence in Zika-affected areas	Microcephaly , intracranial calcifications, ocular abnormalities, hearing loss, neurological deficits	Serology: IgM and IgG antibodies against Zika virus; PCR: Detection of Zika virus RNA in amniotic fluid, cord blood, or tissue	Positive Zika virus-specific IgM and/or IgG antibodies ; Positive Zika virus RNA detection
Congenital Cytomegalovirus (CMV) Infection	No specific epidemiological clue	Hearing loss , intellectual disability, vision impairment, seizures, neurological abnormalities	Serology: IgM and IgG antibodies against CMV; PCR: Detection of CMV DNA in urine, saliva, or cerebrospinal fluid	Positive CMV-specific IgM and/or IgG antibodies; Positive CMV DNA detection
Congenital Toxoplasmosis	Exposure to infected cats or undercooked meat	Chorioretinitis , hydrocephalus, intracranial calcifications, neurological impairment	Serology: IgM and IgG antibodies against Toxoplasma gondii; PCR: Detection of T. gondii DNA in amniotic fluid, cord blood	Positive T. gondii-specific IgM and/or IgG antibodies; Positive T. gondii DNA detection
Congenital Rubella Syndrome	Recent maternal rubella infection	Cataracts , deafness, heart defects, intellectual disability	Serology: IgM and IgG antibodies against rubella virus	Positive rubella-specific IgM and/or IgG antibodies
Congenital Syphilis	Maternal syphilis infection	Skin rash, hepatosplenomegaly, anemia, neurological abnormalities	Serology: VDRL test, RPR test, and treponemal tests (FTA-ABS, TPPA)	Positive VDRL, RPR, FTA-ABS, or TPPA tests
Fetal Alcohol Spectrum Disorders (FASD)	Maternal alcohol consumption during pregnancy	Facial abnormalities , growth deficits, neurodevelopmental disorders	No specific laboratory tests; diagnosis based on clinical findings and maternal history	Clinical findings consistent with FASD
Genetic or Chromosomal Disorders	Family history, genetic testing	Various physical and intellectual disabilities	Karyotype analysis, chromosomal microarray analysis, and specific gene mutation testing	Abnormal karyotype or genetic mutations

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ZIKA VIRUS DISEASE

Signs and symptoms of Zika virus (ZIKV) infection are nonspecific and can resemble other infections. Among these, dengue virus infection is the most serious and potentially life-threatening.

Disease	Dengue	CHIK	Zika
Fever	+++	+++	+++
Myalgia	+++	+++	++
Arthralgia	+++	+++	++
Periarticular Oedema	–	–	++
Maculopapular exanthema	–	++	+++
Retro-orbital pain	++	+	++
Conjunctivitis	–	+	+++
Hepatomegaly	–	+++	–
Bleeding	+	–	–

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4811063/>

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ZIKA VIRUS DISEASE

Comparing Zika virus infection with some diseases prevalent in India, with a focus on their differential diagnosis:

Disease	Zika Virus Infection	Malaria	Dengue Fever	Chikungunya	Japanese Encephalitis	Japanese B Encephalitis
Vector	Aedes mosquitoes (Aedes aegypti, Aedes albopictus)	Anopheles mosquitoes	Aedes mosquitoes	Aedes mosquitoes	Culex mosquitoes	Close contact
Symptoms	Fever, rash, joint pain, conjunctivitis	Fever, chills, sweating, headache	High fever, severe headache, joint pain	Fever, joint pain, rash, headache	Fever, headache, convulsions	Rash, fever, swollen lymph nodes
Pregnancy	Congenital birth defects (microcephaly) possible	Not a major concern in pregnancy	Not a major concern in pregnancy	Not a major concern in pregnancy	Not a major concern in pregnancy	Congenital defects
Diagnosis	RT-PCR for viral RNA, serology (IgM/IgG)	Blood smear	NS1 antigen, IgM/IgG	Serology, RT-PCR	Serology, RT-PCR	Serology, RT-PCR

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ZIKA VIRUS DISEASE

Notes

- **Pregnant Women:** Special attention is given to pregnant women due to the risk of congenital Zika syndrome.
- **Newborns and Infants:** Testing should be considered for infants born to mothers with positive or inconclusive Zika virus test results, as well as infants with microcephaly or other congenital malformations

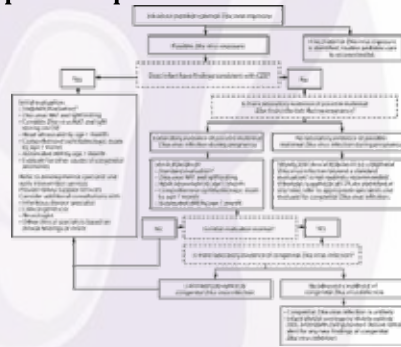


Figure source: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5668694/>

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ZIKA VIRUS DISEASE

If initial test results are negative but Zika virus infection is still suspected, follow-up testing may be required.

In some cases, additional imaging (such as ultrasound or MRI) may be recommended for pregnant women or newborns to check for Zika-related abnormalities.

Proper diagnosis of Zika virus infection is essential for patient management and for taking appropriate public health measures to prevent the spread of the virus.

Center for Drug Modeling, NCI/NCI, Bethesda

ZIKA VIRUS DISEASE

- **Reverse Transcription Polymerase Chain Reaction (RT-PCR)** is the primary method for detecting Zika virus RNA in serum, urine, or other body fluids during the first two weeks after the onset of symptoms. It is most effective within the first 7 days of symptom onset but can sometimes detect the virus in urine up to 14 days post-symptoms.

- **IgM Antibody Testing:** IgM antibodies usually develop toward the end of the first week of illness and can persist for several months.
- **Plaque Reduction Neutralization Test (PRNT):** This test is used to measure virus-specific neutralizing antibodies to differentiate Zika virus infection from other flavivirus infections (like dengue, yellow fever, and West Nile viruses).

Center for Drug Modeling, NCI/NCJ, Bethesda

ZIKA VIRUS DISEASE

Parameter	Details
Type of Sample	Serum
Volume of Sample	About 500 µl or available quantity of serum. Higher volume is preferred.
Storage (0-48 hours)	Refrigerate at 2-8 °C
Storage (48 hours - 7 days)	Freeze at -10 to -20 °C
Storage (>7 days)	Freeze at -70 °C for long-term preservation

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ZIKA VIRUS DISEASE

[illegible]

Guidelines for specimen Collection

- A BSL-2 containment level is required to handle suspected samples.
- Consider all specimens as potentially hazardous/ infectious.
- Handle all specimens with gloves in a secure manner.
- Place each specimen into a separate container labeled with the patient's name and identification number, the collection site, the date of collection and the time of the collection.
- Do not contaminate the outside of the specimen container.
- Do not handle laboratory requisition forms with gloves.

Centre for One Health, NCDC, MoHFW

ZIKA VIRUS DISEASE

Sample Collection

Collecting body fluids other than serum or CSF (e.g., urine, amniotic fluid)

- Provide 0.5–1.0 mL of the specimen in a sterile screw capped vial secured with a small piece of thermoplastic, self-sealing lab film.
- Please ensure a tight seal as leaking specimens cannot be accepted.
- For RNA NAT testing, specimens should be kept cold (2–8 °C) or frozen (≤ 20 °C) for storage and shipping.
- For virus isolation testing, specimens should be frozen as soon as possible (≤ 20 °C).
- Store remainder of sample at ≤ -70°C for long term storage.
- If collecting both urine and semen, obtain the urine specimen first.

Collecting serum or cerebrospinal fluid (CSF)

- At least 1.0 mL* of serum or CSF is required for testing.
- Transfer serum or CSF to a plastic tube measuring approximately 50 mm tall and 15 mm in diameter (e.g., 1.8 mL cryotube or 2.0 mL microtube) with screw cap.
- For virus isolation testing, specimens should be frozen as soon as possible (≤ 20 °C).
- For antibody and RNA NAT testing, specimens should be kept cold (2–8 °C) or frozen (≤ 20 °C).
- Store remainder of sample at ≤ -70°C for long term storage

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Materials are needed for sample collection

Step	Supplies
Safety materials	Appropriate PPE, waste disposal and disinfectants
Sample materials	Swabs, transport medium, suitable collection tools and containers
Suitable labelling materials	Marker pens or barcodes
Triple packaging materials	Absorbent material, plastic bag, sturdy outer container, racks, cooler box, thermometer
Transportation	Regular schedule and method to move the samples to the laboratory, personnel trained in sample handling
Biosafety facility	BSL 2 facility

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Information to be sent to the Apex laboratories

- a) Every sample should be accompanied by appropriate data form
b) NOTE: Following details are must in the data form:

Parameter	Details
Date of Onset of Symptoms	Record the exact date when symptoms began.
Date of Specimen Collection	Record the date the sample was collected for diagnosis.
Pertinent Travel History	Note any travel within 3 months prior to symptom onset.
Female Patient Details	Include details of LMP (last menstrual period) and pregnancy status, if any.
Packaging	Ensure proper triple-layered packaging for transport according to biosafety standards.

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Notes

Guidelines for Transporting Clinical Samples

Parameter	Details
Temperature for Transport	Samples to be sent on ice (maintain +2 to +8 °C).
Sample Labeling and Documentation	Original samples must be packed, labelled, marked, and documented as Category B.
Packaging Requirements	Use standard triple-layered packing as per guidelines for Category B samples.
Prior Notification	Sender must provide prior intimation about sample shipment to RCVRDL.

Note: In case you have any logistic problem or suggestion, kindly contact NIV, Pune

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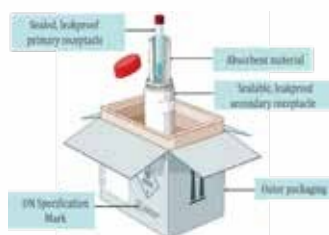
Triple packaging system

The packaging consists of three layers as follows -

Primary receptacle: A labelled primary watertight, leak-proof receptacle containing the specimen. The receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage.

Secondary receptacle: A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.

Outer shipping package: The secondary receptacle is placed in an outer shipping package which protects it and its content from outside influences such as physical damage and water while in transit



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Sample registration and Sample Handling

The sample registration will be done in LIMS, which is the prerequisite for further processing.

Sample Handling and Maintenance:

Sample handling and maintenance team should be supervised by senior staff.

Each sample should be divided into three aliquots.

Aliquots should be stored with different preservatives: EDTA, magnesium sulfate, and decontamination buffer.

The note of "Date opened" should be recorded on the aliquot by a qualified team member.

Sample aliquoting for future research purposes should be prioritized.

Standard aliquoting procedure should be followed.

After aliquoting, the original sample should be discarded.

SNo	Name	Sample sent Form	NIV ID	Aliquot 1	Aliquot 2	Aliquot 3	ELISA 20 ul	PCR 140 ul	Isolation 200 ul
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Sampling Window

Time Frame	Sample Type	Diagnostic Tests
Day 1 - Day 5 after symptom onset	Serum	RT-PCR (Standard & Quantitative) targeting NS5 genomic region
Day 1 - Day 10 after symptom onset	Serum	RT-PCR (Standard & Quantitative) for Zika Virus RNA detection
Day 1 - Day 10 after symptom onset	Urine	RT-PCR (if further validation studies are available for urine samples)
Day 1 - Day 10 after symptom onset	Saliva	RT-PCR (if further validation studies are available for saliva samples)

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Notes

Diagnostic RT-PCR



Category	Details
Diagnostic Method	Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) targeting the non-structural protein 5 (NS5) genomic region.
Method Type	Standard and Quantitative RT-PCR
Advantages	Rapid, specific, and sensitive method for early ZIKV detection.
Sample Types	Serum, Urine, Saliva
Detection Timeline	- Viral RNA detected in serum up to day 10 after symptom onset. - ZIKV RNA detected in urine and saliva samples.
Recommended Sampling	- Serum samples within the first 5 days after symptom onset for optimal results. - Further studies required for urine or saliva samples.
RT-PCR Standardization	Standardized based on published primers at NIV.

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Detection of IgM antibodies to Zika virus



Convalescent Phase Testing (> 5 Days):

- Serology:
- IgM antibodies are tested in blood.
- Not the primary diagnostic method due to high cross-reactivity with other flaviviruses.

Plaque Reduction Neutralization Test (PRNT)*:

- Used for confirmatory diagnosis.
- Highly specific for differentiating ZIKV from other flaviviruses.

Documentation: Test readings and interpretations are to be maintained in a file with test number and date for easy reference.

***PRNT (plaque reduction neutralization assay) is done to confirm or rule out Zika virus infection following a positive, equivocal or inconclusive Zika IgM ELISA result.**

Virus Isolation: Viral isolation is not regarded as a diagnostic tool and is recommended only for supplemental research studies in public health surveillance.

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Choosing the Right Test

The selection of the most appropriate test depends on several factors, including:

- **Symptoms:** Presence or absence of symptoms and their timing.
- **Time since suspected exposure:** NAATs are preferred for recent infection.
- **Pregnancy status:** Pregnant women with suspected Zika infection may require specific testing strategies.

Limitations of Laboratory Diagnosis

- **NAATs (Nucleic Acid Amplification Tests)** have a limited window of detection.
- **Serological tests** can yield positive results for other flaviviruses, necessitating confirmatory testing.
- **Early and accurate diagnosis** is crucial for proper management of the infection, particularly for **high-risk groups**, such as pregnant women.

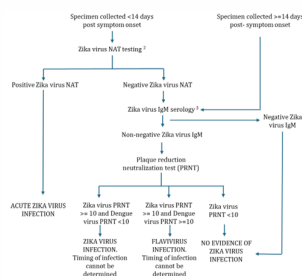
(Refer Ministry of Health & Family Welfare guidelines- <https://www.mohfw.gov.in/Document/Ministry-alerts/technical-guidelines-zika-virus-disease?action=show&manage-zika-virus-disease>)

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Testing Recommendations for Symptomatic Non- Pregnant Individuals with Exposure to Zika Virus (WHO)

- Viral nucleic acid detection remains the preferred method for diagnosis.
- Paired serum and urine are the primary diagnostic specimens for Zika virus infection
- For symptomatic persons, Zika virus RNA can sometimes be detected early in the course of illness using r-RT PCR testing.
- PCR testing should be performed on serum, urine, or CSF samples collected within the revisit two weeks and up to 12 weeks after symptom onset. Urine and CSF specimens must be accompanied by serum.



*NAT testing is not recommended for specimens obtained >14 days post-symptom onset.
†Compatible specimens for NAT testing include serum, or paired matched serum and urine. Repeat NAT testing of a positive result is not indicated. Dengue and Chikungunya virus NAT testing should be performed for patients at risk of concurrent or closely overlapping diseases.
‡Dengue IgM serology should also be performed for patients at risk of exposure, and with clinically compatible illness.

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Notes

Algorithm for Zika Virus Diagnosis, National Institute of Virology, Pune

The clinical samples which are negative for Dengue and Chikungunya should be screened for ZIKV diagnosis.

Standard RT-PCR and quantitative RT-PCR provide a rapid, specific and sensitive method for ZIKV early detection.

Zika virus is diagnosed using PCR (polymerase chain reaction) and virus isolation from blood samples.

Diagnosis by serology can be difficult as the virus can cross-react with other flaviviruses such as Dengue, West Nile and Yellow fever (WHO)

Viral RNA has been detected in serum up to day 10 after the onset of symptoms.

ZIKV RNA also has been detected in urine or saliva samples. However, and since more studies are needed, it is recommended that the serum sample be taken during the first 5 days after the onset of symptoms.

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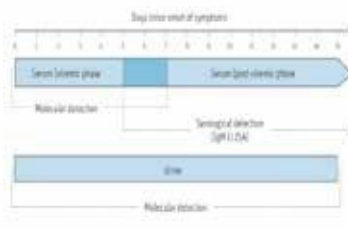
Laboratory Diagnosis

Samples for routine diagnostic- Serum, Urine, microcephaly- CSF
Pregnant women- Amniotic fluid

The virus has been detected in **serum most often within 5 days of symptoms onset**, and, in some cases, longer.

In **urine**, however, **high viral loads have been detected for a long time after the onset of symptoms**

To improve diagnostic sensitivity, it is recommended that **serum and urine samples be obtained at the same time (no later than 15 days after the onset of symptoms)** for processing by RT-PCR

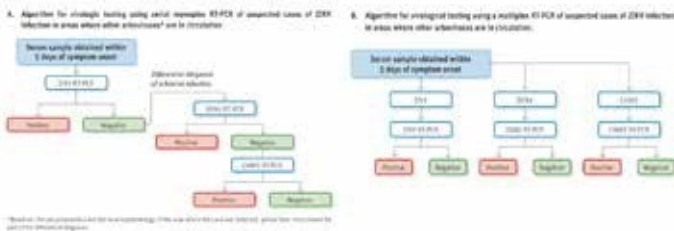


Recommended diagnosis based on number of days since onset of symptoms and sample type

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Diagnostic Algorithm for Differential Diagnosis by RT-PCR



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Diagnostic Algorithm for Differential Diagnosis using ELISA

Sample:- Serum

ELISA is the recommended serology method to detect specific anti-ZIKV IgM antibodies as of day 6 of the onset of symptoms.

Early diagnosis determining or excluding ZIKV infection based on a single serum sample taken during the acute phase is presumptive.

Therefore, it is recommended that a second sample be taken one to two weeks after the first, to rule out infection.

cross-reactivity has been documented with related flaviviruses, especially dengue or yellow fever (53-55). Additionally, PRNT testing is relatively complex and time-consuming.

Serological Diagnosis is not recommended for ZIKV



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Notes

Other Laboratory Tests

Method	Findings
Additional Testing	- Matched serum and urine specimens should be tested simultaneously - RT-PCR can be performed on amniotic fluid, CSF, and placental tissue (not routine)
Serologic Testing	- Enzyme immunoassays, immunofluorescence assays, and neutralization assays are recommended - Cross-reactivity with dengue and other flaviviruses should be considered

Currently, 2 labs in India NCDC (Delhi) and NIV (Pune) have the capability of diagnosing the disease in acute febrile stages

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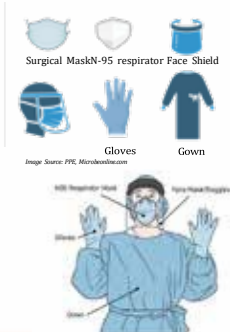
ZIKA VIRUS DISEASE

Notes

What PPE is recommended during infectious specimen handling?

PPE is recommended during infectious specimen collection

- Long-sleeved isolation gown, scrub suits, or coveralls with sleeves that fully cover your torso and extend from your neck to your knees and cover your arms to the end of your wrists. These should close on the backside
- Head coverings, shoe covers or dedicated shoes
- N95 respirator that is secured properly and fits your face
- Eye protection (e.g. goggles or disposable full-face shield) that can be adjusted to fit your face
- Clean non-sterile disposable gloves that cover the wrist area of your isolation gown



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List of Laboratories for PRNT

Plaque Reduction Neutralization Tests (PRNT) for Zika virus diagnosis are performed at highly specialized laboratories that have the necessary biosafety facilities and expertise. Some of the key laboratories equipped for PRNT include:

- National Institute of Virology (NIV), Pune**
 - An apex virology research institute under the Indian Council of Medical Research (ICMR).
 - Performs confirmatory PRNT testing for Zika virus and other arboviruses.
- Regional Medical Research Centres (ICMR RMRCs)**
 - Some RMRCs, especially those focusing on arboviral diseases, may be equipped to perform PRNT under ICMR directives.
- National Centre for Disease Control (NCDC), Delhi**
 - Operates as a central reference lab and may coordinate confirmatory testing or facilitate PRNT through other labs.
- ICMR-designated Virus Research and Diagnostic Laboratories (VRDLs)**
 - Selected VRDLs with advanced facilities may perform PRNT, especially in cases of arboviral outbreaks.

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Global Zika Virus Laboratory Testing Status

Nucleic acid amplification tests (NAAT) are the preferred diagnostic method for detecting viral genomic material, offering confirmed evidence of infection.

Despite the specificity of molecular testing, rare cases of false positive NAAT results have been reported, influenced by the type of assay and patient population characteristics.

Negative NAAT does not exclude recent Zika infection. Therefore, Zika virus immunoglobulin (IgM) antibody testing is recommended in specific situations, as IgM levels rise after symptom onset and persist for up to 12 weeks.

Cross-reactivity with other flaviviruses complicates Zika virus antibody testing, leading to more common false-positive results with IgM compared to NAAT.

PRNTs are quantitative assays that can resolve false-positive IgM results by measuring virus-specific neutralizing antibody titers, providing confirmation against Zika and other flaviviruses.

Confirmatory PRNT is currently available through limited state health departments and CDC, and its use is recommended for definitive diagnosis in certain situations.

In regions with high prevalence of dengue and Zika virus, PRNT may not confirm a significant proportion of IgM positive results, prompting informed decisions about its utility based on local prevalence and performance

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ZIKA VIRUS DISEASE

Session-3

Vector & Vector Management

Course No. 003	Session 3: Vector & Vector Management
Duration	30 minutes
Learning Objective	• Describe vector associated with Zika infection, its lifecycle, and prevalence • Describe Vector Surveillance and its importance • Integrated Vector Management (IVM) approach in India
Pre-requisite knowledge/skill	• Basic knowledge of mosquito biology and vector-borne disease transmission. • Familiarity with entomological surveillance techniques and data interpretation. • Understanding of principles and practices of Integrated Vector Management (IVM).
Competencies to be achieved	• Understanding of vector, its lifecycle and transmission. • Understanding vector surveillance and IVM approach

VECTOR

Aedes aegypti, is a species of mosquito with significant implications for public health. Adult female mosquitoes of this species are known to bite both humans and animals, as they require blood to facilitate the production of eggs. After obtaining a blood meal, female mosquitoes seek out water sources to deposit their eggs.

Notably, both *Ae. aegypti* and *Ae. albopictus*, another mosquito species, have limitations in their flight capabilities, as they are unable to cover long distances. *Ae. aegypti* mosquitoes display a preference for residing in close proximity to human populations, where they actively seek opportunities to bite people. On the other hand, *Ae. albopictus* bites both humans and animals and can be found in diverse environments, both indoors and outdoors.

Breeding Sites

In the Indian context, these mosquito species, particularly *Ae. aegypti*, are prevalent and pose a significant threat, especially in urban areas. The widespread distribution of these mosquitoes is attributed to several factors, including deficient water management practices, solid waste management the presence of non-degradable items such as tires, water coolers, flower pots and durable plastic containers that serve as breeding sites, the rapid expansion of urban areas, and challenges faced by public health authorities in mobilizing communities to address the elimination of mosquito breeding grounds. *Aedes* mosquitoes can bite multiple times.

The primary habitats for these mosquitoes, including *Ae. aegypti*, often includes overhead tanks, ground water storage tanks, and septic tanks. Additionally, natural larval habitats such as tree holes, leaf axles, and coconut shells contribute to their breeding grounds. The population of these mosquitoes tends to fluctuate based on environmental factors such as rainfall and humidity.

In rural areas, the presence of these mosquitoes is associated with the expansion of rural water supply schemes and other development projects that lack proper health impact assessments. This highlights the need for comprehensive strategies to address the breeding grounds of *Aedes* mosquitoes, particularly in rapidly growing urban areas and regions undergoing development without adequate consideration for public health consequences. Understanding the ecological factors influencing their prevalence is crucial for effective mosquito control and the prevention of mosquito-borne diseases.

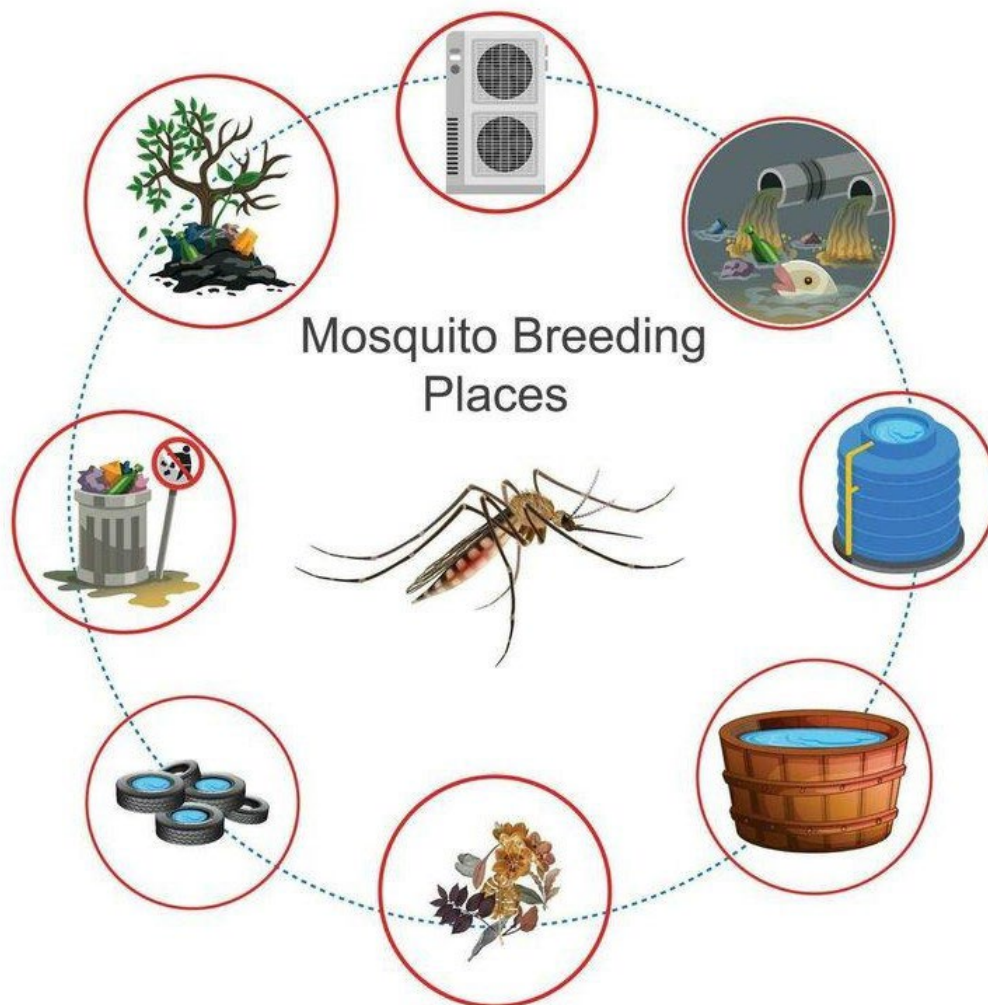


Fig 11: Mosquito breeding sites

Life Cycle of *Aedes aegypti*

Aedes mosquitoes undergo a life cycle consisting of four stages: egg, larva, pupa, and adult. This entire process, from egg to adult mosquito, spans approximately 8-10 days. The life stages of *Aedes* mosquitoes are as follows:

Eggs: Female mosquitoes lay their eggs on the inner wet surfaces of containers with water, above the waterline. Typically, mosquitoes deposit around 100 eggs at a time. These eggs are highly resilient, adhering to container walls like glue and surviving drying out for up to 8 months. A minimal amount of water is sufficient to attract a female mosquito, and various items like bowls, cups, fountains, tires, barrels, and vases storing water become ideal breeding sites.

Larva: Larvae emerge from mosquito eggs only when the water level rises to cover the eggs. Rainwater or human additions triggering an increase in water level prompt the larvae to emerge. Larvae feed on microorganisms present in the water and undergo three molting stages before transforming into pupae.

Pupa: Pupae undergo development until the newly formed adult mosquito emerges from the pupal skin and leaves the water.

Adult: Upon emergence, male mosquitoes feed on nectar from flowers, while female mosquitoes feed on humans and animals to obtain blood necessary for egg production.

After feeding, female mosquitoes seek out water sources to lay additional eggs.

Aedes aegypti, a specific species of *Aedes* mosquitoes, has limited flight range, flying only a few blocks during its lifespan.

Unlike other mosquito species, *Aedes aegypti* mosquitoes prefer biting humans. *Aedes aegypti* mosquitoes favor living in close proximity to people, often found inside homes, buildings, and businesses where window and door screens are absent, or doors are left open.

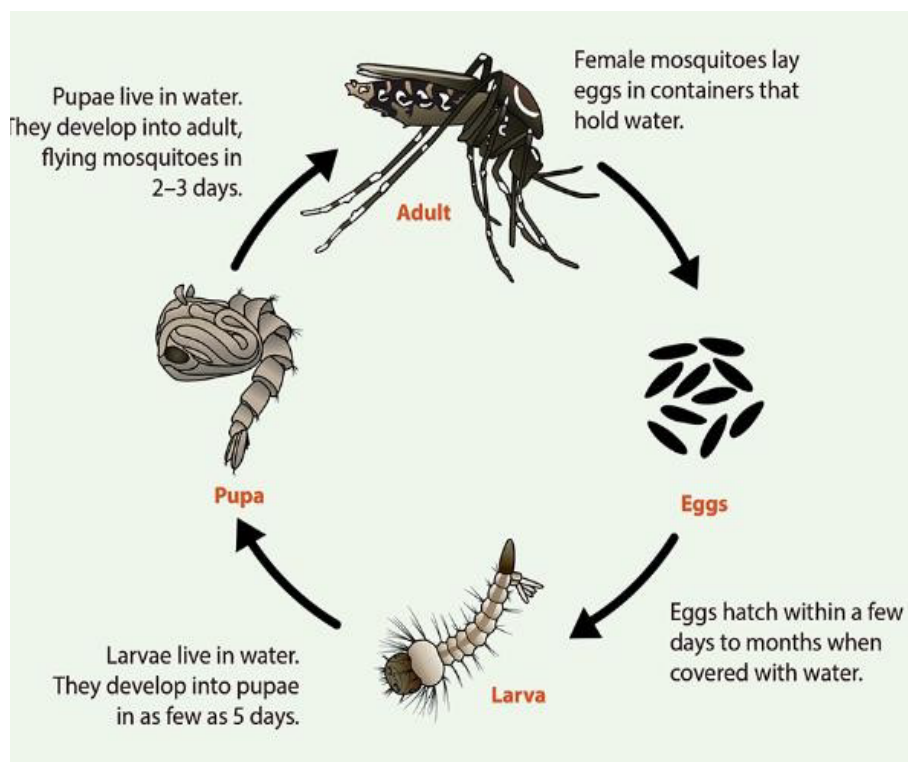


Fig 12: Life Cycle of *Aedes aegypti* (CDC)

VECTOR SURVEILLANCE

Entomological surveillance plays a crucial role in the success of vector control initiatives. Rigorous monitoring at entry points is particularly vital for countries facing the potential introduction of *Aedes* mosquitoes. For areas with established population, monitoring of *Aedes* at immature and adult life stages should be undertaken. For immatures, it is essential to know the key containers in a given settings as studies have shown that as few as 3-5 container types can sustain 80% of the total pupal population in an area. This requires an assessment to quantify larval and/or pupal populations across all containers in the area. Knowledge of the spatial distribution, density and resting site of adults are also important if this stage will be targeted for control. The species composition and their susceptibility to insecticides either in use or planned for use should also be determined. This information will facilitate appropriate and timely decision-making for interventions (*WHO Vector control operations framework for Zika virus*).

Given the widespread distribution of vectors, it is crucial to implement a comprehensive strategy involving regular vector surveillance and integrated management of *Aedes* mosquitoes through both biological and chemical control methods that are safe, cost-effective, and environmentally friendly. This approach also encompasses environmental management, legislation, and actions at the household and community levels.

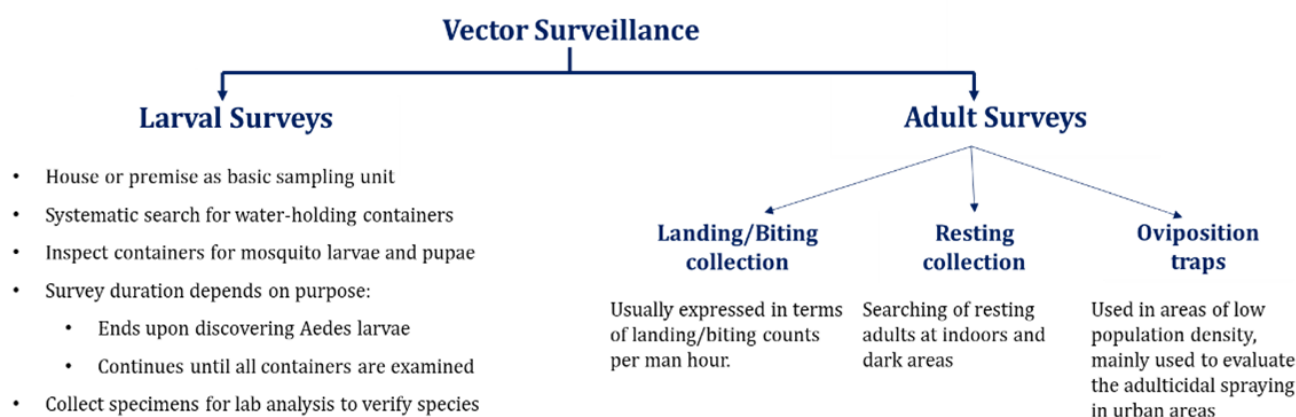


Fig 13: Vector Surveillance and Control

Larval Surveys

The fundamental sampling unit is the house or premise, systematically searched for containers holding water. Inspection of containers is conducted to detect the presence of mosquito larvae and pupae. The survey's duration depends on its objective; it may conclude upon finding *Aedes* larvae or persist until all containers are thoroughly examined. Specimens are collected for laboratory analysis to confirm the mosquito species. Four indices that are used to monitor the *Ae aegypti* infection levels are -

1. **House Index (HI):** Percentage of houses infected with larvae and/or pupae

$$HI = \frac{\text{Number of houses infected}}{\text{Number of houses inspected}} \times 100$$

2. **Container Index (CI):** Percentage of water holding containers infected with larvae or pupae

$$CI = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

3. **Breteau Index (BI):** Number of positive containers per 100 houses inspected

$$BI = \frac{\text{Number of positive containers}}{\text{Number of houses inspected}} \times 100$$

4. Pupa Index (PI): Number of pupae per 100 houses

$$CI = \frac{\text{Number of pupae}}{\text{Number of houses inspected}} \times 100$$

Adult Surveys

- Landing/Biting Collection: Typically expressed as landing or biting counts per man-hour.
- Resting Collection: Involves the search for resting adults in indoor and dark areas.
- Oviposition Traps: Utilized in areas with low population density, primarily for evaluating adulticidal spraying in urban settings.

INTEGRATED VECTOR MANAGEMENT (IVM) APPROACH

Integrated Vector Management (IVM) is a systematic decision-making process aimed at optimizing the utilization of resources for controlling disease-carrying vectors. This approach enhances the efficiency, cost-effectiveness, ecological soundness, and sustainability of interventions against vectors, utilizing the available tools and resources. By focusing on multiple Vector-Borne Diseases (VBDs), IVM significantly impacts public health more than singular disease-focused control programs. The integrated strategy of IVM is adaptable to various VBDs.

IVM transforms traditional vector control systems by making them more evidence-based, integrated, and participative. However, its successful implementation necessitates changes in the roles and responsibilities of stakeholders and institutional mechanisms. Key elements of IVM include advocacy, social mobilization, reinforcing regulatory and legislative controls for public health, empowering communities, and fostering collaboration within health and other sectors during planning, decision-making, resource utilization, and strategy implementation.

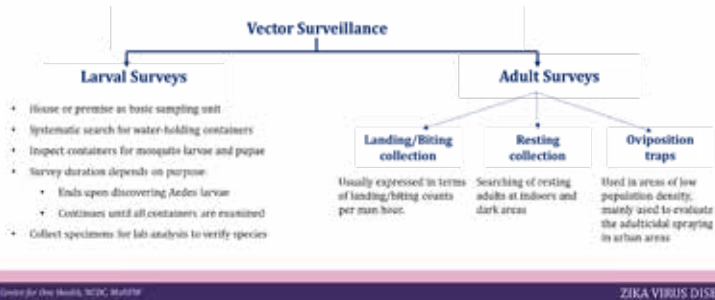
Planning and implementing IVM involve assessing epidemiological and entomological situations at the country level, analyzing local disease determinants, determining suitable vector control measures, assessing requirements and available resources, and designing effective implementation strategies. Skilled staff and adequate infrastructure at both central and local levels are crucial for successful IVM implementation.

The fundamental concepts for IVM implementation include:

- Which mosquito species are locally important as vectors of human diseases?
- Which mosquito species are important as the primary source of annoyance?
- What are the important breeding sites of different mosquito species?
- What is the seasonal pattern of mosquito breeding?
- What are the resting places of adult mosquitoes?
- What are the feeding preferences of vector mosquitoes?
- To map out and locate all potential breeding sites
- To identify the mosquito/sand fly species present
- To predict the time and location of effective control strategies

Vector Surveillance and Control

Considering the widespread presence of vectors, it is crucial to implement a comprehensive strategy for regular vector surveillance. This strategy should include the integrated management of *Aedes* mosquitoes through safe and cost-effective biological and chemical control measures. Additionally, environmental management, legislation, and action at both household and community levels are essential components of this approach.



Indices Used in Monitoring *Aedes aegypti* Infection

1. House Index (HI): Percentage of houses infected with larvae and/or pupae

$$HI = \frac{\text{Number of houses infected}}{\text{Number of houses inspected}} \times 100$$

2. Container Index (CI): Percentage of water holding containers infected with larvae or pupae

$$CI = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

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Indices Used in Monitoring *Ae aegypti* Infection (continued)

3. Breteau Index (BI): Number of positive containers per 100 houses inspected

$$BI = \frac{\text{Number of positive containers}}{\text{Number of houses inspected}} \times 100$$

4. Pupae Index (PI): Number of pupae per 100 houses

$$PI = \frac{\text{Number of pupae}}{\text{Number of houses inspected}} \times 100$$

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Integrated Vector Management (IVM)

- Which mosquito species are locally important as vectors of human diseases?
- Which mosquito species are important as a primary source of annoyance?
- What are the important breeding sites?
- What is the seasonal pattern of mosquito breeding?
- What are the resting places and feeding preference of mosquito?
- Mapping of potential breeding sites
- Prediction of time and location of effective control strategies

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ZIKA VIRUS DISEASE

Notes

Session-4

Treatment, Prevention and Case Study

Course No. 004	Session 4: Treatment & Prevention and Case Study
Duration	30 minutes
Learning Objective	• Describe a sample case of Zika Virus Disease • Add one case for pregnant lady as well • Understand treatment options • Describe preventive measures
Pre-requisite knowledge/skill	• Basic understanding of clinical symptoms and case management in viral diseases. • Familiarity with antenatal care protocols and risks of congenital infections. • Awareness of standard public health prevention strategies for mosquito-borne diseases.
Competencies to be achieved	• Comprehension of the case study and general understanding of handling of Zika cases • Explanation of strategies for treatment and prevention of Zika Virus Disease

TREATMENT

Zika virus infection poses a unique challenge in the medical field, as there is currently no specific treatment available for the disease. When individuals exhibit symptoms such as rash, fever, or joint pain, the recommended course of action is centered around supportive care. Patients are advised to prioritize rest, maintain adequate hydration, and manage their symptoms through the use of antipyretics and/or analgesics.

One crucial aspect of managing Zika virus infection lies in the careful selection of medications. Aspirin and other Nonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided, particularly in the early stages of the illness. This precaution is rooted in the need to rule out concurrent dengue virus infections, as the use of NSAIDs in such cases may increase the risk of bleeding complications. The careful consideration of medication choices underscores the importance of precise and tailored medical interventions for patients affected by Zika virus.

Special attention is warranted for pregnant women, especially those residing in areas with active Zika transmission. Pregnant individuals who develop symptoms indicative of Zika infection are strongly advised to seek prompt medical attention. Beyond symptomatic relief, laboratory testing becomes essential in confirming the presence of the virus. Additionally, these women are entitled to comprehensive information, counseling, and appropriate clinical care to address the unique risks and implications of Zika virus infection during pregnancy. The emphasis on early detection and targeted care highlights the critical role of healthcare providers in managing Zika cases, particularly in the context of maternal and fetal health.

The National Blood Transfusion Council (NBTC) plays a pivotal role in safeguarding the integrity of the blood supply in the country, especially in the context of emerging threats such as Zika virus infection. To prevent the transmission of Zika through blood transfusion, blood banks are strictly mandated to adhere to stringent donor selection criteria and robust screening processes. One key measure is the thorough assessment of an individual's travel history, particularly focusing on those returning from regions experiencing Zika outbreaks. Donors with recent travel to such areas are barred from contributing blood for a period of 120 days post-return. Moreover, these potential donors must remain asymptomatic during this interval to ensure the safety of the blood supply.

In addition to travel-related precautions, blood banks are directed not to accept blood from donors until a minimum of two weeks after the complete recovery from any acute viral infection, with the cessation of all relevant therapies and medications. This precautionary period is crucial in mitigating the risk of viral transmission through donated blood. Individuals who have returned from outbreak zones are not only subject to deferred donation but are also urged to promptly report any signs and symptoms of Zika infection to the blood bank within two weeks of their donation.

It is noteworthy that, as of the advisory, the NBTC has not endorsed any specific blood donor screening tests for Zika virus. This underlines the need for a comprehensive approach that includes thorough donor evaluation and history-taking, as well as a reliance on symptoms and temporal considerations. Furthermore, to maintain the safety of the blood supply, the importation of human plasma from regions or countries affected by Zika outbreaks is strictly prohibited. This comprehensive set of guidelines underscores the NBTC's commitment to ensuring the highest standards of blood safety and preventing the inadvertent transmission of Zika virus through the blood transfusion process.

PREVENTION

The primary strategies for preventing Zika virus transmission encompass a multifaceted approach. Firstly, efforts to prevent transmission from mosquito is essential for Zika virus disease prevention. This involves implementing rigorous mosquito control measures, including the elimination of breeding sites and the use of insecticides. Community engagement and public awareness campaigns play pivotal roles in promoting personal protective measures, such as wearing long sleeves and using mosquito repellent.

Another crucial aspect of prevention involves addressing sexual transmission. Individuals, especially those residing in or traveling from Zika-affected areas, are advised to practice safe sex or abstain from sexual activities to reduce the risk of sexual transmission. This precautionary measure is particularly important, given the ability of Zika virus to persist in semen for an extended period.

Pregnant individuals or those planning pregnancy are encouraged to take proactive steps to prevent Zika infection during pregnancy. This includes avoiding travel to areas with active Zika transmission and practicing strict mosquito bite prevention measures.

Blood screening is an essential component of Zika virus prevention to mitigate the risk of transfusion-related transmission. Rigorous screening of donated blood helps identify and exclude potentially contaminated blood units, safeguarding the blood supply and reducing the risk of transmission through blood transfusions. These multifaceted prevention strategies collectively contribute to mitigating the impact of Zika virus, protecting individuals and communities from the associated health risks. Public health initiatives, education, and collaboration between healthcare providers and communities are integral to the successful implementation of these preventive measures.

Vector Management

Ae aegypti breeds mostly in man made receptacles found in and around households, factories, construction sites, etc., Natural larval habitats are rare, but include tree holes, leaf axles and coconut shells. The population of this mosquito fluctuates with rainfall and humidity. Under optimal conditions, the life cycle of the aquatic stage (hatching to adult emergence) would be as

short as seven days. However, it takes several weeks at low temperatures. Therefore, during the rainy season when survival is under optimum condition, the risk of virus transmission is greater.

The rural spread of *Aedes* is, however, majorly associated with expansion of development projects without proper health impact assessments, scarcity of water with consequent water storage, improper use of water coolers and disposable containers and improper solid waste management. The effective control and management of vector include the following components (*NCVBDC Guidelines for IVM of Aedes mosquito*) –

Environmental Management

The major methods used for control of *Aedes* mosquito are as follows –

- a. **Environmental modification:** Long lasting physical transformation of vector habitats, such as – improved water supply, mosquito proofing of overhead tanks, cisterns or underground reservoirs, etc.
- b. **Environmental manipulation:** Temporary changes to vector habitats that involve the management of essential and non-essential containers and management of or removal of natural breeding sites.
- c. **Changes in human habitations:** Efforts are made to reduce man-virus contact by mosquito proofing of houses with screen on doors/windows.

Personal Protection

Protective clothing and repellents are common means of personal protection against mosquitoes and biting insects. Household insecticide products, such as mosquito coils, pyrethrum space spray and aerosols have been used extensively for personal protection against mosquitoes. Insecticide treated mosquito nets have limited utility on Zika control, as the mosquito bite mostly during daytime. However, insecticide treated bed nets can be effectively used to protect infants and night workers while sleeping in daytime.

Biological Control

Larvivorous fish (*Gambusia*, Mosquitofish, Bluegill fish) are recommended in large water bodies or large water containers. Endotoxin producing bacteria, such as *Bacillus thuringiensis* serotype H-14 has been found an effective mosquito control agent.

Chemical Control

Chemical control, such as larvicides, adulticides, are recommended in permanent big water containers where water has to be conserved or stored because of scarcity of water or irregular and unreliable water supply.

Since *Ae aegypti* breeds in clean household water, only larvicides that are safe, without any odour or color, with residual effect with low mammalian toxicity and without any health hazards, should be used. Considering the criteria, Temephos, an organophosphate compound is used under public health programs with the recommended dose of 1 ppm (1 mg per liter of water). Pictures of chemical control.

For the control of adult mosquitoes, Pyrethrum spray may be used in indoor situations. Additionally, Malathion fogging or Ultra Low Volume (ULV) spray are used where a cloud of insecticide droplets remains suspended in the air for a long time.

Legislative Measures

Suitable laws and byelaws should be enacted and implemented for regulating storage/utilization of water by communities and industries.

- a. **Model civic byelaws:** If breeding is detected, penalties or fines are imposed. This has been strictly enforced by Mumbai, Navi Mumbai, Chandigarh, and Delhi Municipal Corporations.
- b. **Building Construction Regulation Act:** Regulations governing building bylaws should include specifications for suitable overhead/underground tanks, structures that are mosquito-proof, and the design of features like sunshades and porticos. In Mumbai, before commencing any construction, property owners or builders are required to submit a fee to the Municipal Corporation for the purpose of managing conditions conducive to mosquito breeding at the construction site.
- c. **Environmental Health Act:** Suitable byelaws should be made for the proper disposal/storage of junk, discarded tins, old tyres, and other debris, which can withhold rainwater.
- d. **Health Impact Assessments:** Appropriate legislation should be formulated for mandatory HIA prior to any development projects/major constructions.

Prevention of Mosquito bites

In the context of insect repellent use, it is recommended to employ Environmental Protection Agency (EPA)-registered insect repellents for an effective protection against mosquitoes. When applying repellent to babies and children, adults should follow specific precautions, including dressing the child in clothing covering arms and legs and using mosquito netting to cover strollers and carriers. It is crucial to follow label instructions meticulously, with a particular emphasis on avoiding products containing oil of lemon eucalyptus (OLE) or para-menthane-diol (PMD) on children under 3 years old. Repellent application on a child's face should be done by applying it onto the adult's hands first, avoiding sensitive areas.

For everyone, regardless of age, adherence to product label instructions and regular reapplication of insect repellent as directed are paramount. Additionally, it is advised not to apply repellent on skin under clothing, and when using sunscreen simultaneously, apply sunscreen first followed by insect repellent. Regarding natural insect repellents not registered with the EPA, their effectiveness remains uncertain, emphasizing the importance of relying on registered products for proven results.

Further preventive measures include wearing loose-fitting, long-sleeved shirts and pants, treating clothing and gear with 0.5% permethrin, or purchasing permethrin-treated items. Permethrin is an insecticide effective against mosquitoes and maintains its efficacy after multiple washings. It is crucial, however, not to apply permethrin directly on the skin. To control mosquitoes both indoors and outdoors, utilizing screens on windows and doors, repairing screen holes, and employing air conditioning, if available, are recommended. Additionally, taking proactive steps to eliminate or manage water-holding containers, both inside and outside, helps disrupt mosquito breeding cycles, contributing to a comprehensive approach to mosquito-borne disease prevention.

Prevention of Mosquito breeding

In the context of preventing mosquito breeding, it is crucial to implement a range of effective measures to minimize the risk of mosquito-borne diseases. One primary strategy involves covering all water tanks and containers with tight lids to prevent mosquitoes from accessing stagnant water sources for breeding. Additionally, the disposal and destruction of unused containers, junk materials, tires, coconut shells, and other potential breeding sites are essential to eliminate mosquito habitats.

Regular maintenance practices play a vital role in mosquito control. Emptying, scrubbing, and drying desert coolers on a weekly basis before refilling them help disrupt the mosquito breeding cycle. This routine maintenance is critical in preventing the accumulation of stagnant water where mosquitoes lay their eggs. Introducing larvivorous fish in ornamental tanks, both at residential houses and hotels, serves as a natural and environmentally friendly approach to controlling mosquito larvae and curbing their population.

Implementing a weekly dry day is another effective measure in mosquito prevention. During this day, individuals should empty, scrub, and dry all containers holding water, such as bird baths, flower vases, and ant-trappers. This systematic and consistent approach helps eliminate potential breeding grounds for mosquitoes, reducing the risk of vector-borne diseases. Training programs should emphasize the importance of these practices and educate communities on the role each individual plays in maintaining a mosquito-free environment through proactive and regular preventive measures.

Prevention of Sexual Transmission

The prevention of sexual transmission of the Zika virus is a critical aspect of public health, considering that individuals infected with the virus may remain asymptomatic or experience mild symptoms. Additionally, transmission can occur even when infected individuals are not exhibiting any symptoms. To safeguard against the potential spread of Zika through sexual contact, it is imperative to adopt effective preventive measures.

Condom usage is a key component in reducing the risk of Zika transmission during sexual activity. Both male and female condoms are recommended, and for optimal effectiveness, they should be consistently and correctly used throughout the entire duration of vaginal, anal, and oral sex, as

well as during the sharing of sex toys. Dental dams can be employed for specific types of oral sex, such as mouth-to-vagina or mouth-to-anus contact. Not sharing sex toys is an additional precautionary measure to diminish the risk of spreading Zika to sexual partners.

A comprehensive approach to preventing sexual transmission involves emphasizing the importance of abstinence from sexual activity. While condoms and other protective measures are crucial, abstaining from sexual intercourse entirely eliminates the risk of acquiring Zika through sexual contact. These strategies, rooted in scientific understanding, serve as essential guidelines for individuals to protect themselves and their partners from the potential sexual transmission of the Zika virus. Public health campaigns and educational initiatives should prioritize disseminating this information to raise awareness and promote responsible sexual health practices.

Case Scenario 1

Patient Details:

- Age: 28 years
- Gender: Female

Chief Complaints:

- High-grade fever for 4 days
- Generalized body ache for 4 days
- Pain in all joints for 3 days

Identifying Zika Virus Infection: The patient's history reveals recent travel to Kanpur for a family function, a region with a documented outbreak of Zika virus (130+ cases in 2021). Zika virus is transmitted via human-vector-human transmission, making travel history a key factor in diagnosis.

Symptoms Observed:

- Temperature: 38.4°C
- Severe myalgia and arthralgia
- Maculopapular rash with itching
- Nonpurulent conjunctivitis
- Headache and retro-orbital pain

Case Classification:

- **Suspect Case:** Defined by the presence of a skin rash or body temperature >37.2°C and at least one of the following:
 - ◇ Arthralgia or Myalgia
 - ◇ Nonpurulent conjunctivitis or conjunctival hyperemia
 - ◇ Headache or Malaise
 - ◇ History of travel to areas with indigenous transmission of Zika Virus in the last two weeks.

- **Confirmed Case:** A suspect case with a laboratory-confirmed positive RT-PCR result for Zika Virus.

Diagnosis:

- The patient meets the suspect case criteria due to fever, rash, associated symptoms, and recent travel history.
- RT-PCR for Zika Virus from blood and urine samples confirmed the infection.

Management and Preventive Measures:

- Supportive treatment:
 - ◊ Adequate rest and hydration
 - ◊ Acetaminophen for fever management
- Preventive measures:
 - ◊ Safe sex practices for at least 2 months post-recovery (for women) and 3 months (for men) to prevent sexual transmission.
 - ◊ Vector control measures to prevent mosquito bites.

Key Takeaways:

- Zika virus presents with nonspecific symptoms similar to other arboviral infections, making history-taking crucial for diagnosis.
- Laboratory confirmation with RT-PCR is necessary to establish a definitive diagnosis.
- Management is primarily supportive, with a strong emphasis on preventive measures.
- Public health awareness and vector control play a crucial role in limiting the spread of the disease.

Case Scenario 2

Patient Details:

- Age: 28-year-old married female (married for 2 years)

Chief Complaints:

- High-grade fever for 4 days
- Rash over the body for 4 days
- Pain in all joints for 3 days

Important History:

- Recent travel outside India to a country experiencing a Zika virus outbreak.
- Amenorrhoea (3 months), conjunctival congestion, and similar symptoms in the husband are important history details.
- Zika infection during pregnancy requires special consideration due to potential fetal defects.

Differential Diagnoses to Consider:

- Dengue Fever
- Malaria

- Chikungunya
- SARS-CoV-2
- Chickenpox
- Mpox (Monkeypox)
- West Nile Fever
- Viral Hepatitis
- Leptospirosis
- Scrub Typhus
- Influenza
- Japanese Encephalitis
- All these conditions present as Acute Febrile Illnesses, potentially associated with rash.

Exclusions:

- No cough or breathlessness → Excludes viral respiratory illness.
- No jaundice → Excludes hepatitis or leptospirosis.
- Japanese encephalitis may present with neurological symptoms later.

Investigations for Zika Virus Infection:

- Primary diagnostic methods:
 - ◊ RT-PCR is the preferred method for detecting Zika Virus RNA in serum, urine, or other body fluids during the first two weeks.
 - ◊ PRNT assay is performed to confirm or rule out Zika Virus infection if IgM Zika result is positive.
- Testing in pregnant women:
 - ◊ Asymptomatic pregnant women in areas with active Zika transmission should be tested for IgM antibodies as part of routine obstetric care.
 - ◊ Symptomatic pregnant women should first be tested for Dengue and Chikungunya before testing for Zika.
- Explanation for Zika Testing:
 1. Diagnosis can be challenging due to cross-reactivity with other Flaviviruses.
 2. PRNT helps confirm Zika Virus if the IgM test is positive.

Investigation Plan:

Arrange the investigations in the proper order:

- Fetal ultrasound -----5
- IgM for Dengue and IgM for Chikungunya----- 2
- Zika Virus IgM testing by ELISA -----4
- Pregnancy testing ----- 1
- Zika Virus NAT testing----- 3

Diagnosis:

- The patient meets the suspect case criteria due to fever, rash, associated symptoms, and recent travel history.
- RT-PCR for Zika Virus confirmed the infection.

Management and Preventive Measures:

- Supportive treatment:
 - ◊ Adequate rest and hydration
 - ◊ Acetaminophen for fever management
- Preventive measures:
 - ◊ Safe sex practices for at least 2 months post-recovery (for women) and 3 months (for men) to prevent sexual transmission.
 - ◊ Vector control measures to prevent mosquito bites.

Key Takeaways:

- Zika virus presents with nonspecific symptoms similar to other arboviral infections, making history-taking crucial for diagnosis.
- Laboratory confirmation with RT-PCR is necessary to establish a definitive diagnosis.
- Management is primarily supportive, with a strong emphasis on preventive measures.
- Public health awareness and vector control play a crucial role in limiting the spread of the disease.

- Individuals experiencing symptoms such as rash, fever, or joint pain should rest, stay hydrated, and manage symptoms (Acetaminophen (paracetamol))
- It is important to avoid nonsteroidal anti-inflammatory drugs until dengue virus infections are ruled out due to the risk of bleeding.
- Prescribe medicine such as acetaminophen to reduce fever and pain.
- If a person is taking medicine for another medical condition, she or he should talk to their healthcare provider before taking additional medication.
- Pregnant women living in areas with Zika transmission or those who develop symptoms should seek medical attention for laboratory testing, information, counseling, and appropriate clinical care.



There is no specific medicine or vaccine for Zika virus.

- There is no vaccine to prevent Zika virus disease.
- **Main Zika virus prevention strategies include:**
- Preventing Mosquito Transmission
- Preventing Sexual Transmission
- Preventing Zika Infection During Pregnancy
- Prevention during blood and organ transfusion



Situation Analysis

Epidemiological
Assessment

Vector Assessment

Stratification

Local Determinants of Disease

Selection of Vector Control Methods

Chemical based

Non-chemical based

Needs & Resources

Understanding of available and required resources for the planned activity. Intersectoral coordination and resource sharing are recommended

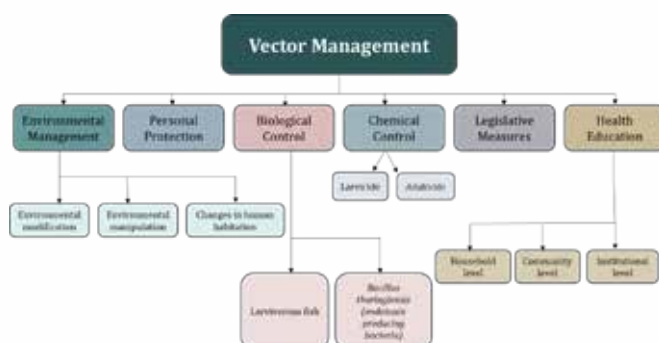
Implementation

Adherence to SOPs

Safe Handling and Disposal

Monitoring & Evaluation

Indicator based Monitoring & Evaluation process is required to better understand the output of the designed plan



Health Impact Assessments: Appropriate legislation should be formulated for mandatory HIA prior to any development projects/major constructions.

ZIKA VIRUS DISEASE

- ## Personal Protection

- ZIKA VIRUS DISEASE

01

- Consistent and correct use of condoms (male or female) is critical during vaginal, anal, and oral sex.
- Dental dams can be used for oral contact (mouth-to-vagina or mouth-to-anus).

02

- Do not share sex toys to prevent the spread of the virus.

03

- Abstaining from sexual activity eliminates the risk of sexual transmission.

04

- Public health campaigns should educate individuals on the risks of Zika transmission through sexual contact.

ZIKA VIRUS DISEASE

- Preventive measures include using insect repellents, wearing long-sleeved shirts and pants, and eliminating mosquito breeding sites such as standing water.
- It is important to prevent mosquitoes from entering homes by using screens on windows and doors.
- Individuals who have travelled to areas with known Zika virus transmission should take precautions to avoid mosquito bites for several weeks upon returning home to prevent local transmission.
- Pregnant women are advised to avoid traveling to areas with Zika virus circulation due to the association with microcephaly. Guidelines for testing pregnant women exposed to Zika virus are available through the CDC.
- Infected individuals should refrain from donating blood for several months.
- Currently, there is no approved vaccine against Zika virus



ZIKA VIRUS DISEASE

[illegible]

National Blood Transfusion Council (NBTC) advisory for blood banks and potential blood donors to prevent Zika virus infection in the country by blood transfusion

• **Donor Selection and Screening:**

- Strict adherence to donor selection criteria.
- Mandatory travel history check for potential donors.

• **Post-Travel Blood Donation Restrictions:**

- Individuals returning from outbreak zones should not donate blood for 120 days post-return.
- Donors must be symptom-free during this period.

• **Post-Infection Blood Donation Restrictions:**

- No blood donation for 2 weeks following complete recovery from any acute viral infection and cessation of medication.

• **Reporting Symptoms:**

- Donors developing Zika symptoms within 2 weeks post-donation must report to the blood bank.



• **Import Restrictions:**

- No human plasma imports from Zika outbreak regions/countries.

• **Screening Tests:**

- No approved blood donor screening tests for Zika virus by NBTC.

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ZIKA VIRUS DISEASE

Scenario 1

Patient Presentation

- Patient Details: 28-year-old female

• Chief Complaints:

- High-grade fever (4 days)
- Generalized body ache (4 days)
- Pain in all joints (3 days)



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ZIKA VIRUS DISEASE

Identifying Zika Virus Infection

Which history makes you suspect Zika virus infection?

- a) She has cattle at home
- b) She had recently travelled to Kanpur for a family function
- c) She was scratched by her pet dog
- d) She received a tetanus toxoid injection for the same

• **Correct Answer: b) Travel to Kanpur**

Explanation:

- Kanpur had 130+ Zika cases in 2021.
- Zika is transmitted via human-vector-human transmission.

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ZIKA VIRUS DISEASE

Symptoms Observed

• **Findings on Examination:**

- Temperature: 38.4°C
- Severe myalgia & arthralgia
- Maculopapular rash with itching
- Nonpurulent conjunctivitis
- Headache and retro-orbital pain

• Question: Which of these symptoms are common in Zika virus disease?

• Answer: **All the above.**

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ZIKA VIRUS DISEASE

Explanation

Ref : Case Classification

Suspect Case – Skin rash or body temp $> 37.2^{\circ}\text{C}$ & One of the following symptoms

- * Arthralgia or Myalgia
- * Nonpurulent conjunctivitis or conjunctival hyperaemia
- * Headache or Malaise
- * History of travel to countries/places with indigenous transmission of Zika Virus in last 2 weeks

Confirmed Case : Suspect case & Lab positive result for Zika Virus by RT-PCR

Ref: Clinical Features of Zika Virus Infection

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ZIKA VIRUS DISEASE

Classification of Zika Virus Disease

Question 1: Is this a suspect case of Zika?

Answer: **Yes/No**

Explanation:

- Suspect case criteria: Fever or rash + other symptoms + travel history.

Question 2: Is this a confirmed case of Zika?

Answer: **Yes/No**

Explanation:

- Confirmed case requires positive RT-PCR result.

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ZIKA VIRUS DISEASE

Management and Preventive Measures

She was advised to give blood and urine sample, and her RT PCR for Zika Virus disease was positive.

Question: Which statements are true?

- a) Adequate rest and hydration
- b) Oseltamivir (Incorrect)
- c) Diclofenac for pain (Incorrect)
- d) Acetaminophen for fever
- e) Safe sex for 2 months post-recovery

Correct Answers: a, d, e

Explanation:

- Treatment for Zika Virus infection is mainly supportive including adequate rest, hydration and acetaminophen for fever.
- Oseltamivir is given for H1N1 infection.
- Preventive measures include prevention of mosquito bites and safe sex practices for at least 2 months by women and 3 months by men, as the disease can be transmitted by sexual contact.

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ZIKA VIRUS DISEASE

Scenario 2

Patient Presentation

• Patient Details

Age: 28-year-old married female
(married for 2 years)

• Complaints:

- Fever (high grade) – 4 days
- Rash over body – 4 days
- Pain in all joints – 3 days



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ZIKA VIRUS DISEASE

Notes

Important History

Travel history outside India to a country with a Zika virus outbreak.

Which of the following history details are important?

- a) Amenorrhoea (3 months)
- b) Conjunctival congestion
- c) Husband has similar symptoms
- d) Hypothyroidism (taking Tablet Eltroxin)

Explanation: Zika infection during pregnancy requires special consideration due to fetal defects.

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ZIKA VIRUS DISEASE

Differential Diagnoses to Consider

Dengue Fever	Malaria	Chikungunya
SARS-CoV-2	Chickenpox	Mpox (Monkeypox)
West Nile Fever	Viral Hepatitis	Leptospirosis
Scrub Typhus	Influenza	Japanese Encephalitis

• All these are Acute Febrile Illnesses which may be associated with rash.

• **Exclusions:**

- No cough or breathlessness → Excludes viral respiratory illness.
- No jaundice → Excludes hepatitis or leptospirosis.
- If patient has Japanese encephalitis, then neurological symptoms may occur later.

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ZIKA VIRUS DISEASE

Investigations for Zika Virus Infection

Which of the following are true?

- a) RT-PCR is the primary method for detecting Zika Virus RNA in serum, urine or other body fluids during first 2 weeks
- b) IgM antibodies testing is more specific and should be done in all suspected cases
- c) All asymptomatic pregnant women should be tested for Zika Virus infection.
- d) All asymptomatic pregnant women residing in areas with active Zika transmission should be tested for IgM antibodies as a part of routine obstetric care.
- e) Symptomatic pregnant women should be tested for Dengue and Chikungunya first.
- f) PRNT assay is done to confirm or rule out Zika Virus infection if IgM Zika result is positive

Explanation for Zika Testing:

1. Diagnosis can be difficult due to cross-reactivity with other Flaviviruses.
2. PRNT helps confirm Zika Virus if IgM test is positive.

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ZIKA VIRUS DISEASE

Investigation Plan

Arrange the investigations in the proper order:

- Foetal ultrasound ----- 5
- IgM for Dengue and IgM for Chikungunya ---- 2
- Zika virus IgM testing by ELISA----- 4
- Pregnancy testing----- 1
- Zika Virus NAT testing ----- 3

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ZIKA VIRUS DISEASE

Notes

Session-5

Outbreak Management & Response

Course No. 005	Session 5: Outbreak Management & Response
Duration	15 minutes
Learning Objectives	• Describe outbreak management and case classification • Understand outbreak situation in India • Understanding public health scenario • Describe outbreak response and preparedness • Describe collaboration between healthcare providers and public health authorities
Pre-requisite knowledge/skill	• Basic knowledge of outbreak investigation principles and case classification. • Familiarity with national disease surveillance and reporting systems. • Understanding of intersectoral coordination in public health emergencies.
Competencies to be achieved	• Understanding potential sources of outbreak • Describing reported Zika outbreaks in India • Understanding roles of departments in combatting outbreaks • Explain measures to improve outbreak response and preparedness • Competency in effectively managing outbreaks of Zika

OUTBREAKS IN INDIA

The first serological evidence of Zika Virus outbreak dates back to 1954. Notable outbreaks occurred in 2017 in states such as Gujarat, Rajasthan, and Kerala, followed by further outbreaks in Madhya Pradesh and Uttar Pradesh in 2018. More recently, in 2021, Kerala, Uttar Pradesh, and Maharashtra experienced Zika outbreaks. In 2016, the World Health Organization (WHO) declared Zika a Public Health Emergency of International Concern (PHEIC) due to its link to severe birth defects. In response to these outbreaks, India implemented a range of surveillance and control measures, including expanding sentinel surveillance networks, conducting nationwide vector surveillance for both Zika and Dengue, and initiating newborn birth defect screening programs in tertiary hospitals.

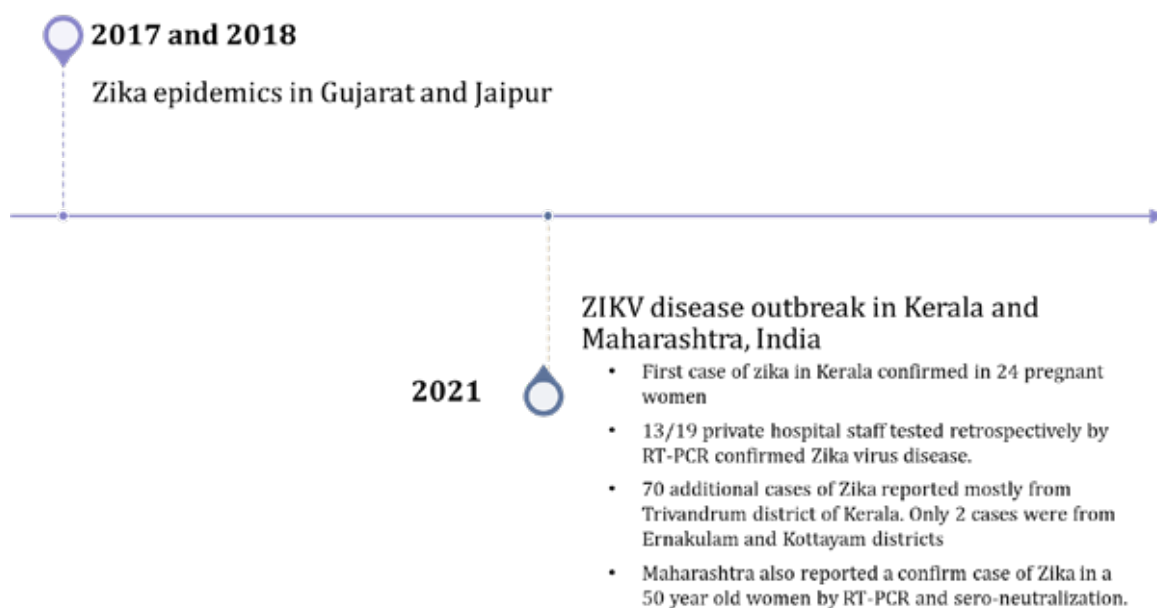


Fig 14: Recent Zika virus outbreaks in India

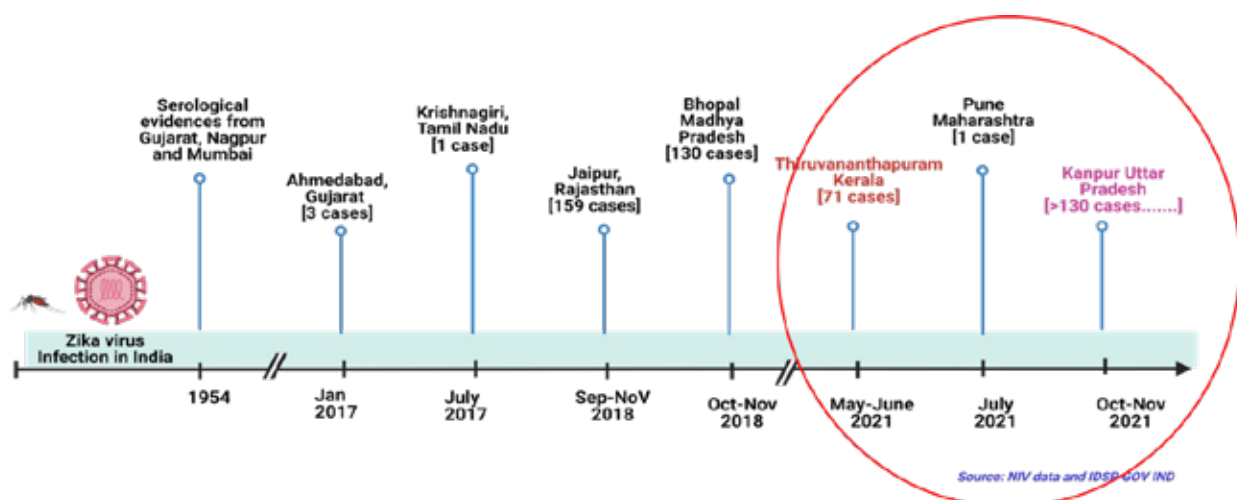


Fig 15: Timeline of Zika virus disease outbreaks in India

CLUSTER CONTAINMENT STRATEGY

A comprehensive cluster containment strategy for Zika virus disease should include the following key components:

Rapid Response Team Activation

A dedicated team should be activated to coordinate response activities, including surveillance, case management, vector control, and community engagement.

Intensified Surveillance

Enhanced active and passive surveillance to identify new cases and monitor disease trends.

Vector Control

Implementation of targeted vector control measures, such as larviciding, adulticiding, and source reduction, to reduce mosquito populations.

Case Management

Provision of prompt medical care to affected individuals, including supportive treatment and management of complications.

Community Engagement and Risk Communication

Effective communication with the community to raise awareness about Zika virus, its transmission, and preventive measures.

Laboratory Testing

Timely and accurate laboratory testing to confirm Zika virus infections.

Surveillance of Pregnancy Outcomes

Close monitoring of pregnant women for any signs of Zika virus infection and potential fetal complications.

Data Management and Analysis

Regular data collection, analysis, and reporting to inform decision-making and resource allocation.

CLUSTER CONTAINMENT PLAN FOR ZIKA VIRUS DISEASE OUTBREAK

Daily Report Format

Date:

State:

District:

Block:

Epicentre:

A. Population-Based Information

Parameter	Daily	Cumulative
No. of Villages/Municipalities/Localities Surveyed		
Population Surveyed		
Population Surveyed within 0-3 km Radius		

B. Morbidity Data

Parameter	Daily	Cumulative
Persons with Fever/Symptoms Consistent with Zika Virus Disease (0-3 km)		
Persons with Acute Flaccid Paralysis (0-3 km)		
Pregnant Women with Fever/Symptoms Consistent with Zika Virus Disease (0-3 km)		

C. Hospital-Based Information

Parameter	Daily	Cumulative
Outpatient Visits (Fever Cases)		
Inpatient Admissions (Fever Cases)		
Inpatient Admissions (Acute Flaccid Paralysis)		
Pregnant Women Suspected of Zika Virus Disease		

D. Monitoring Health Staff

Category	Number Deployed	Number with Fever/Symptoms
Field Staff (Medical Officers, Health Supervisors, Health Workers)		
Hospital Staff (Medical Officers, Nurses, Attendants)		

E. Stock Position

Item	Previous Day's Stock	Consumption	Stock on Hand	Requisition (if any)
Pyrethrum Extract 2%				
Malathion Technical				
Cyphenothrin 5% EC				
PPE				
N-95 Masks				

The Integrated Disease Surveillance Project (IDSP) underscores the importance of a systematic approach to outbreak investigation. This involves a series of steps that health professionals can follow to effectively identify, investigate, and control disease outbreaks.

OUTBREAK INVESTIGATION RESPONSE AND CONTROL

Detecting an outbreak is a critical step in public health response. Information from various sources, including:

- Media reports
- Key community informants
- Routine surveillance data can signal an impending outbreak.

Key indicators to watch for include:

- Clustering of cases or deaths in time and/or space
- Unusual increase in the number of cases or deaths
- Single cases of diseases like measles, AFP, cholera, plague, dengue, or JE
- Acute febrile illness of unknown etiology
- Occurrence of two or more epidemiologically linked cases of meningitis, measles
- Unusual isolates
- Shifting in age distribution of cases
- Sudden increase/high vector density
- Natural disasters

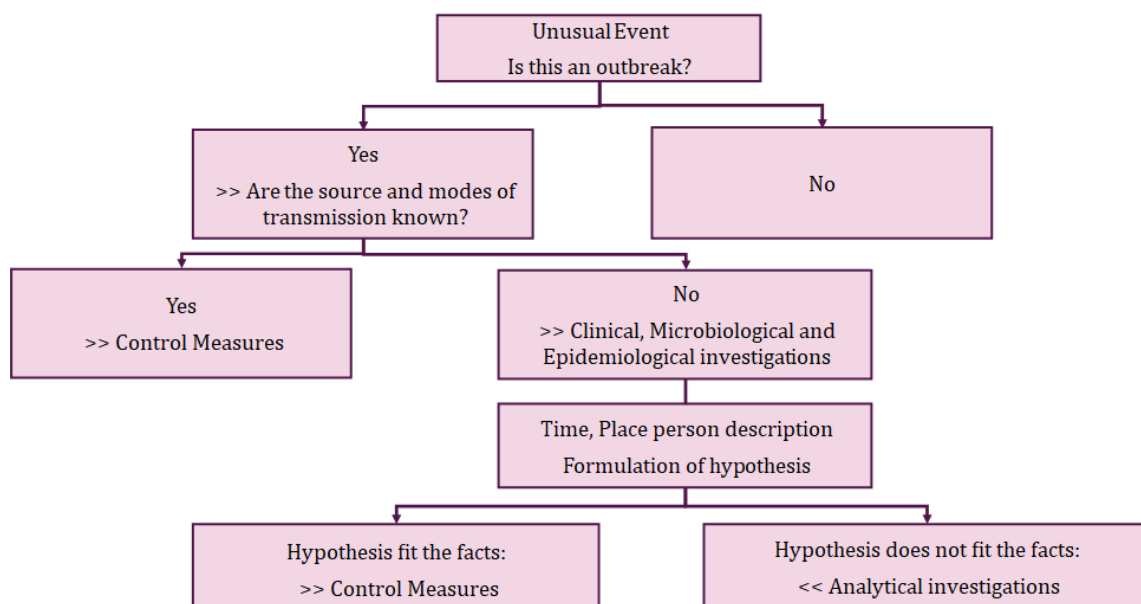


Fig 16: Outbreak Investigation

(Source: IDSP)

Response and Control Measures

Once an outbreak is suspected or confirmed, a multi-level response is initiated:

Level	Actions	Key Responsibilities
Local Level (MO and DSO)	Verification: Confirm the outbreak through epidemiological and laboratory investigations.	Ensure accurate confirmation of the outbreak.
	Initiate Control Measures: Implement immediate control measures based on the specific disease and local context.	Address immediate public health threats.
	Report to DSO: Provide regular updates on the situation and control measures.	Maintain consistent communication with higher authorities.
District Level (DSO and DEIT)	Analysis: Analyze outbreak data to identify trends and risk factors.	Use epidemiological data to inform response strategies.
	Notification: Notify program officers and CMO for coordination of resources and actions. Alert Adjacent Districts: Inform neighboring districts and CHCs to enhance surveillance and preparedness.	- Facilitate timely communication with key stakeholders. - Ensure a regional approach to outbreak management.
State Level (SSO)	Analysis: Review outbreak data to identify broader trends and potential implications.	Assess statewide implications and strategies.
	Appropriate Action: Allocate resources, deploy rapid response teams, and issue public health advisories as needed.	Coordinate a comprehensive and effective outbreak response at the state level.

Trigger Levels for Outbreak Response

To streamline the response to outbreaks, trigger levels can be defined based on the severity and potential impact of the disease. These levels can help guide the appropriate level of response and resource allocation.

- **Trigger Level 1: Suspected/Limited Outbreak:** Local response by health workers and Medical Officers.
- **Trigger Level 2: Outbreak:** Local and district response by DSO and DEIT.
- **Trigger Level 3: Confirmed Outbreak:** Local, district, and state-level response.
- **Trigger Level 4: Widespread Epidemic:** State-level response to an epidemic.
- **Trigger Level 5: Disaster Response:** Local, district, state, central, and partner organizations.

In endemic regions, changes in disease patterns or clusters of cases should be considered warning signs.

Timely action is essential to contain outbreaks. The First Information Report (FIR) should be submitted to the District Surveillance Officer as soon as a suspected outbreak is verified. This can be done through various means, including phone, fax, email, or online data entry.

To be prepared for outbreaks, several measures should be taken:

- **Forming a dedicated Disease Early Warning System (DEIT)** to coordinate response efforts.
- **Training DEIT members** to ensure they are equipped to handle outbreaks.
- **Regularly reviewing surveillance data** to identify trends and anomalies.
- **Identifying outbreak-prone seasons and regions** to focus resources and preparedness efforts.
- **Ensuring adequate supplies of essential drugs, materials, and transport** in outbreak-prone areas.
- **Strengthening laboratory capacity** for timely diagnosis.
- **Maintaining communication channels** to facilitate rapid information sharing and coordination.

Steps in Outbreak Response

Step	Key Actions	Details
Step 1: Verification	1. Identify Validity of Source of Information	Confirm accuracy to avoid false alarms or data errors.
	2. Consult with the Medical Officer (MO)	Assess abnormal increase in cases, clustering, epidemiological links, trigger events, or multiple deaths.
	3. Alert Neighbouring Districts/ Health Centres	DSO informs nearby districts, CHCs, and PHCs to implement preventive measures.
	4. Notify Relevant Authorities	DSO informs Programme Officer and CMO for further actions.
	Rapid Response Teams (DEIT)	Composition: Epidemiologist, clinician, microbiologist, other specialists as needed.
		Roles: Investigate, assist local health staff, follow-up.
		Contact Info: Updated contact details available with DSO and State Surveillance Officer.
Step 2: Sending DEIT	1. Constitute DEIT	Form a team based on outbreak type.
	2. Orientation	Brief DEIT on the current situation.
	3. Resource Allocation	Ensure vehicles, drugs, reagents, and forms are available for fieldwork.
	Investigation Protocol	File interim report to DSO/Programme Officer/CMO.
		- Conduct follow-up visits. - Initiate special studies if outbreak persists.
Step 3: Monitoring	Continuous Updates	DEIT updates DSO on trends in cases/deaths, containment measures, drug/vaccine stocks, logistical issues, community involvement, and media response.
	Duration	Monitoring continues until no new cases are reported and outbreak is officially declared over.
Step 4: Declaring Over	Criteria	No new cases for two incubation periods following the last case.
		Comprehensive active case search confirms no new cases.
Step 5: Final Report	Submission	PHC MO submits a final report within 10 days of outbreak closure.
	Review and Recommendations	Technical Committee reviews report and provides recommendations to prevent future outbreaks.

General Control Measures

Control measures should be initiated immediately upon detecting an outbreak:

1. General Measures:

- Implement interventions even before identifying specific sources or transmission routes. For example, if waterborne disease is suspected, promote safe drinking water usage.

2. Resource Mobilization:

- **Human Resources:** Deploy additional medical officers, lab technicians, and nursing staff.
- **Drugs:** Mobilize necessary medications from other sources.
- **Equipment and Supplies:** Ensure adequate supplies are available for response efforts.
- **Vehicles:** Secure transportation for rapid response teams.

3. Communication Channels:

- Establish 24-hour communication between district officials and outbreak team leaders.

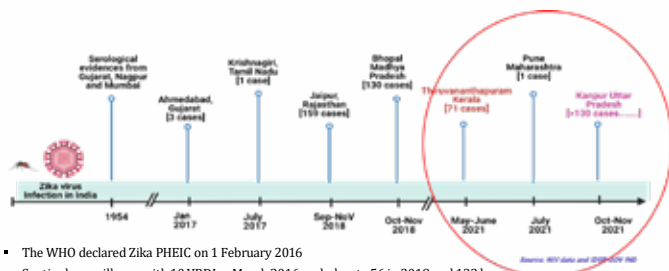
4. Information, Education, and Communication (IEC):

- Educate the community about the outbreak to dispel rumors and misinformation.

5. Media Handling:

- Designate a spokesperson to manage media inquiries regarding the outbreak status to ensure accurate information dissemination.

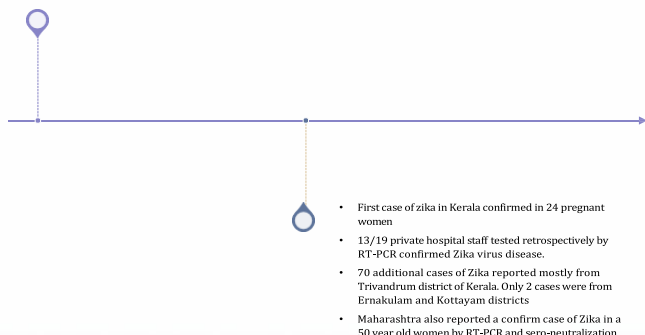
Timeline of Zika virus disease outbreaks in India



- The WHO declared Zika PHEIC on 1 February 2016
- Sentinel surveillance with 10 VRDLs- March 2016 scaled up to 56 in 2018 and 132 by 2021
- Nationwide vector surveillance ZIKV and DENV - 2016-19 [NP Kumar et al 2021]
- Newborn birth defect screening in 55 tertiary hospitals of India

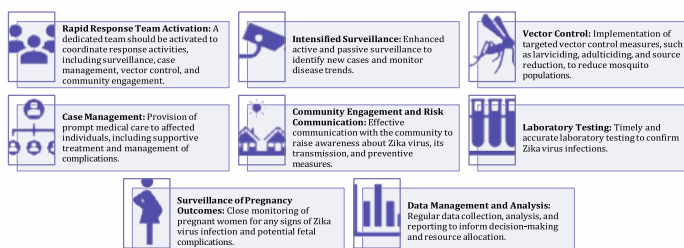
Sophal et al 2018, Yadav et al 2019, Mathura et al 2020

Outbreaks in India



Cluster Containment Strategy

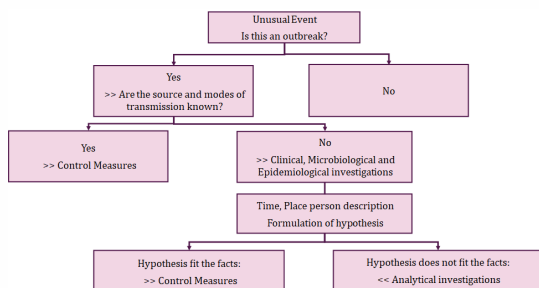
A comprehensive cluster containment strategy for Zika virus disease should include the following key components:



Cluster Containment Plan for Zika Virus Disease Outbreak

Section	Parameter	Daily	Cumulative
A. Population-Based Information	Number of Villages/Municipalities/Localities Surveyed		
	Population Surveyed		
	Population Surveyed within 0-3 km Radius		
	Persons with Fever/Symptoms Consistent with Zika Virus Disease (0-3 km)		
B. Morbidity Data	Persons with Acute Flaccid Paralysis (0-3 km)		
	Pregnant Women with Fever/Symptoms Consistent with Zika Virus Disease (0-3 km)		
	Outpatient Visits (Fever Cases)		
	Inpatient Admissions (Fever Cases)		
C. Hospital-Based Information	Inpatient Admissions (Acute Flaccid Paralysis)		
	Pregnant Women Suspected of Zika Virus Disease		
	Category	Number Deployed	Number with Fever/Symptoms
	Field Staff (Medical Officers, Health Supervisors, Health Workers)		
D. Monitoring Health Staff	Hospital Staff (Medical Officers, Nurses, Attendants)		
	Item	Previous Day's Stock	Consumption
	Pyrethrum Extract 2%		
	Malathion Technical		
E. Stock Position	Cyphenothrin 5% EC		
	PPE		
	N-95 Masks		

Outbreak Investigation



Courtesy: IDSP

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ZIKA VIRUS DISEASE

Outbreak Investigation Response and control

Detecting an outbreak is a critical step in public health response. Information from various sources, including



Media reports



Key community informants



Routine surveillance data can signal an impending outbreak.

Key indicators to watch for include:

- Clustering of cases or deaths in time and/or space
- Unusual increase in the number of cases or deaths
- Single cases of diseases like measles, AFP, cholera, plague, dengue, or JE
- Acute febrile illness of unknown Etiology
- Occurrence of two or more epidemiologically linked cases of meningitis, measles
- Unusual isolates
- Shifting in age distribution of cases
- Sudden increase/high vector density
- Natural disasters

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Response and Control Measures

Once an outbreak is suspected or confirmed, a multi-level response is initiated:

Level	Actions	Key Responsibilities
Local Level (MO and DSO)	- Verification: Confirm the outbreak through epidemiological and laboratory investigations.	- Ensure accurate confirmation of the outbreak.
	- Initiate Control Measures: Implement immediate control measures based on the specific disease and local context.	- Address immediate public health threats.
	- Report to DSO: Provide regular updates on the situation and control measures.	- Maintain consistent communication with higher authorities.
District Level (DSO and DEIT)	- Analysis: Analyze outbreak data to identify trends and risk factors.	- Use epidemiological data to inform response strategies.
	- Notification: Notify program officers and CMO for coordination of resources and actions.	- Facilitate timely communication with key stakeholders.
	- Alert Adjacent Districts: Inform neighboring districts and CHCs to enhance surveillance and preparedness.	- Ensure a regional approach to outbreak management.
State Level (SSO)	- Analysis: Review outbreak data to identify broader trends and potential implications.	- Assess statewide implications and strategies.
	- Appropriate Action: Allocate resources, deploy rapid response teams, and issue public health advisories as needed.	- Coordinate a comprehensive and effective outbreak response at the state level.

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Trigger Levels for Outbreak Response

1	2	3	4	5
Trigger Level 1: Suspected/Limited Outbreak: Local response by health workers and Medical Officers.	Trigger Level 2: Outbreak: Local and district response by DSO and DEIT.	Trigger Level 3: Confirmed Outbreak: Local, district, and state-level response.	Trigger Level 4: Widespread Epidemic: State-level response to an epidemic.	Trigger Level 5: Disaster Response: Local, district, state, central, and partner organizations.

- Timely action is essential to contain outbreaks.
- The First Information Report (FIR) should be submitted to the District Surveillance Officer as soon as a suspected outbreak is verified.
- This can be done through various means, including phone, fax, email, or online data entry.

In endemic regions, changes in disease patterns or clusters of cases should be considered warning signs.

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Notes

Measures for outbreak preparedness



- **Forming a dedicated Disease Early Warning System (DEIT)** to coordinate response efforts.
- **Training DEIT members** to ensure they are equipped to handle outbreaks.
- **Regularly reviewing surveillance data** to identify trends and anomalies.
- **Identifying outbreak-prone seasons and regions** to focus resources and preparedness efforts.
- **Ensuring adequate supplies of essential drugs, materials, and transport** in outbreak-prone areas.
- **Strengthening laboratory capacity** for timely diagnosis.
- **Maintaining communication channels** to facilitate rapid information sharing and coordination.

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	2. Orientation	- Form a team based on outbreak type.
	3. Resource Allocation	- Brief DEIT on the current situation.
	Investigation Protocol	- Ensure vehicles, drugs, reagents, and forms are available for fieldwork.
		- File interim report to DSO/Programme Officer/CMO.
		- Conduct follow-up visits.
		- Initiate special studies if outbreak persists.

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ZIKA VIRUS DISEASE

General Control Measures

Control measures should be initiated immediately upon detecting an outbreak

- **General Measures:** Implement interventions even before identifying specific sources or transmission routes. For example, if waterborne disease is suspected, promote safe drinking water usage.
- **Resource Mobilization:**
 - **Human Resources:** Deploy additional medical officers, lab technicians, and nursing staff.
 - **Drugs:** Mobilize necessary medications from other sources.
 - **Equipment and Supplies:** Ensure adequate supplies are available for response efforts.
 - **Vehicles:** Secure transportation for rapid response teams.
- **Communication Channels:** Establish 24-hour communication between district officials and outbreak team leaders.
- **Information, Education, and Communication (IEC):** Educate the community about the outbreak to dispel rumors and misinformation.
- **Media Handling:** Designate a spokesperson to manage media inquiries regarding the outbreak status to ensure accurate information dissemination.

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ZIKA VIRUS DISEASE

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NOTE TO FACILITATORS

This learning guide has been meticulously designed to enhance the understanding and expertise of doctors, veterinarians, paramedical staff, and laboratory personnel in dealing with Zika Virus Disease cases. Your role is crucial in ensuring the success of this training, so please follow these instructions carefully:

Familiarize Yourself with the Content:

Thoroughly review all the materials provided in the Zika Virus learning guide. This includes presentations, handouts, case studies and assessment tools.

Tailor the Training to Your Audience:

Understand the professional backgrounds of your participants (doctors, veterinarians, paramedics, and lab personnel). Tailor your examples and discussions to make the training relevant to their specific roles.

Set Clear Objectives:

Begin each session by clearly stating the objectives participants are expected to achieve by the end. This will give them a sense of direction and purpose throughout the training.

Create an Interactive Learning Environment:

Encourage active participation and open discussions. Use real-world scenarios to prompt critical thinking and problem-solving.

Use Multimodal Techniques:

Utilize a mix of teaching methods, such as presentations, group discussions and case-based learning, this will cater to different learning styles and keep participants engaged.

Encourage Questions:

Welcome questions and create a safe environment for participants to seek clarifications. This fosters deeper understanding and engagement.

Provide Regular Feedback:

Throughout the training, provide constructive feedback on participants' performance. This will help them identify areas for improvement and enhance their skills.

Summarize and Review:

At the end of each module, summarize key points and takeaways. Conduct a brief review to reinforce learning before moving on to the next topic.

Assessment and Certification:

Develop an assessment mechanism, such as quizzes, practical assessments, or case presentations. Issue certificates of completion to participants who meet the training requirements.

Adapt to Participants' Pace:

Gauge the participants' pace of learning. Adjust the training speed accordingly, ensuring that everyone can follow and grasp the content.

Facilitate Networking:

Create opportunities for participants to interact and network with each other. This can lead to valuable knowledge exchange and collaboration.

Remember, your role as a trainer or facilitator is to guide participants through this comprehensive Zika Virus Disease training with expertise, enthusiasm, and a dedication to enhancing their professional skills. Your efforts will contribute to building a more capable and knowledgeable medical workforce prepared to handle Zika Virus Disease cases effectively.

Course Outline

It is imperative to underscore that the course outline harmonizes with the course schedule. Consequently, any modifications or adaptations made to the course schedule should be duly reflected in this course outline. This synergy between the schedule and the outline ensures consistency and alignment between training plans and their actual implementation.

NOTE: Make copies of the schedule, one for each participant and include in the training material bag.

Time	Topics/Activities	Training/Learning Methods	Resources/Materials
20 min	Welcome participants Facilitate introductions of participants Explore participants' expectations for the course	Open course with a word of welcome by organizers, lead trainers, etc. Facilitate the introductions of all participants and trainers. Explore participants' expectations for the course. Allow participants to freely explore their expectations. In the next session on review of the course objectives, address which expectations can be met and which cannot be met.	Prepare welcome sign, Name badges
20 min	Review course objectives and schedule. Review components of the training guide	Review the course goals and objectives; the course design and expected outcomes; Review the course schedule, including starting and ending times and times for breaks and lunch; Review the materials to be used in the course and ensure that participants understand the use of course materials.	Copies of course schedule, one for each participant from Facilitators' Guide (FG). Full set of the training material : Facilitators' Guide (FG) Questionnaire
20 min	Assess participants' pre-course knowledge	Distribute the Pre-course Knowledge Assessment sheet to each participant. Ask them to answer each question. Allow 15 minutes for assessment. Immediately review the correct responses—Have participants grade the papers while the trainer reviews the correct answers with them and collect the papers after reviewing all the answers.	Pre-Training Quiz Sheet for each participant
2-3 hours	Presentation/ Discussion	Use the PowerPoint slides to present information	Power Point slides on the sessions planned as per agenda
20 min	Assess participants' post training knowledge	Distribute the Post Training Knowledge Assessment sheet to each participant. Ask them to answer each question. Allow 15 minutes for the Post Training Knowledge Assessment.	Copies of Post Training Knowledge Assessment Sheets (one for each participant)
15 min	Course Evaluation	Ask questions of the participants and engage them in the presentation of the information.	
15 min	Closing	Closing remarks by training organizers. Distribute certificates to participants.	Certificate for each participant

PRE/POST TRAINING QUIZ

Instructions for the trainer:

- The trainer/instructor will administer the test before the training and collect the response.
- The same test will be given to the trainee after the completion of the training.
- The difference in scores will be recorded for training evaluation purpose.

Trainee Name:

Date:

Designation:

Test taken before the training (yes/no):

Test taken after the training (yes/no):

Instructions for the trainee:

- The test quiz carries 20 questions
- Attempt all the questions.
- Put a tick mark on the correct option or fill the blanks with True/ False
- Please check whether all questions are answered before submitting

1. Where was the Zika virus first identified?

- a. Brazil
- b. Uganda
- c. French Polynesia
- d. India

2. What is the primary mode of Zika virus transmission to humans?

- a. Foodborne
- b. Airborne
- c. Mosquito bites
- d. Direct contact with infected individuals

3. Which mosquitoes are the main vectors for Zika virus transmission?

- a. Anopheles mosquitoes
- b. Culex mosquitoes
- c. Aedes mosquitoes
- d. Mansonia mosquito

4. What is the primary preventive measure recommended to avoid Zika virus infection?

- a. Wearing sunglasses
- b. Using sunscreen
- c. Applying insect repellent and using protective clothing
- d. Avoiding sexual intercourse

5. **In which region did the Zika virus cause its first large outbreak in 2007?**
 - a. Europe
 - b. Africa
 - c. Pacific island of Yap
 - d. South America
6. **What birth defect is associated with Zika virus infection during pregnancy?**
 - a. Down syndrome
 - b. Autism
 - c. Microcephaly
 - d. Cleft lip
7. **Apart from mosquito bites, how else can Zika virus be transmitted?**
 - a. Food consumption
 - b. Airborne transmission
 - c. Sexual activity
 - d. Casual contact
8. **What is a common characteristic of both *Ae. aegypti* and *Ae. albopictus* in terms of flight capabilities?**
 - a. Long-distance flight ability
 - b. Nocturnal flight preference
 - c. Limited flight capabilities
 - d. High-altitude flight patterns
9. **Where do *Ae. aegypti* mosquitoes, particularly, prefer to reside?**
 - a. Forests and jungles
 - b. Near bodies of water
 - c. In close proximity to human populations
 - d. High-altitude regions
10. **What environmental factors contribute to the fluctuation in the population of *Aedes* mosquitoes, including *Ae. aegypti*?**
 - a. Wind patterns
 - b. Temperature variations
 - c. Soil composition
 - d. Geological features

- 11. How long does the entire life cycle of Aedes mosquitoes, from egg to adult, typically span?**
- a. 2-4 days
 - b. 8-10 days
 - c. 15-20 days
 - d. 30-40 days
- 12. Which indices are used to monitor the infection levels of Aedes mosquitoes, specifically Ae. aegypti?**
- a. Aedes Index (AI) and Mosquito Index (MI)
 - b. Container Index (CI) and Breteau Index (BI)
 - c. Mosquito Index (MI) and Pupae Index (PI)
 - d. Larval Index (LI) and Adult Index (AI)
- 13. Which legislative measure is recommended for the control of Aedes mosquitoes in terms of building construction?**
- a. Zoning restrictions
 - b. Health Impact Assessments (HIA)
 - c. Environmental Impact Assessments (EIA)
 - d. Construction speed limits
- 14. What clinical manifestations of Zika Virus Disease (ZVD) can overlap with other diseases, making accurate diagnosis challenging?**
- a. Respiratory symptoms only
 - b. Gastrointestinal symptoms only
 - c. Neurological symptoms only
 - d. Skin rash, diarrhea, neurological manifestations, and haemorrhagic symptoms
- 15. How is Zika virus diagnosed in the laboratory setting?**
- a. Bone Marrow
 - b. Urinalysis
 - c. Viral culture only
 - d. PCR and virus isolation from blood samples
- 16. What is the primary purpose of the Integrated Disease Surveillance Programme (IDSP) in the context of Zika virus monitoring?**
- a. To provide healthcare services to pregnant women
 - b. To establish quarantine facilities at international airports
 - c. To conduct fever surveillance and report suspected Zika cases
 - d. To coordinate international airline operations

- 17. What role does the Airport/Port Health Organization (APHO/PHO) play in preventing the spread of Zika virus at international airports? ANSWER is d not A.**
- a. Issuing guidelines for aircraft disinfection
 - b. Conducting laboratory tests for Zika virus
 - c. Providing healthcare services to travellers
 - d. Managing quarantine facilities for suspected cases
- 18. What is the recommended course of action for managing Zika virus infection when individuals exhibit symptoms like rash, fever, or joint pain?**
- a. Administer nonsteroidal anti-inflammatory drugs (NSAIDs) for immediate relief
 - b. Prioritize rest, maintain hydration, and use antipyretics/analgesics for symptom management
 - c. Prescribe antibiotics for early intervention
 - d. Recommend immediate hospitalization for all cases
- 19. What is the role of the National Blood Transfusion Council (NBTC) in preventing Zika transmission through blood transfusion?**
- a. Providing medical treatment for Zika-infected individuals
 - b. Ensuring the integrity of the blood supply through donor selection criteria and screening
 - c. Distributing Zika virus vaccines to blood banks
 - d. Conducting research on Zika virus transmission
- 20. What is a key preventive measure to minimize the risk of mosquito-borne diseases, including Zika virus?**
- a. Avoiding mosquito nets and repellents
 - b. Encouraging the accumulation of stagnant water for natural mosquito habitats
 - c. Implementing weekly dry days to empty and scrub water-holding containers
 - d. Using non-registered natural insect repellents

ANSWER KEY

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LIST OF CONTRIBUTORS

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Developed by
Centre for One Health
National Centre for Disease Control
Ministry of Health and Family Welfare
Government of India