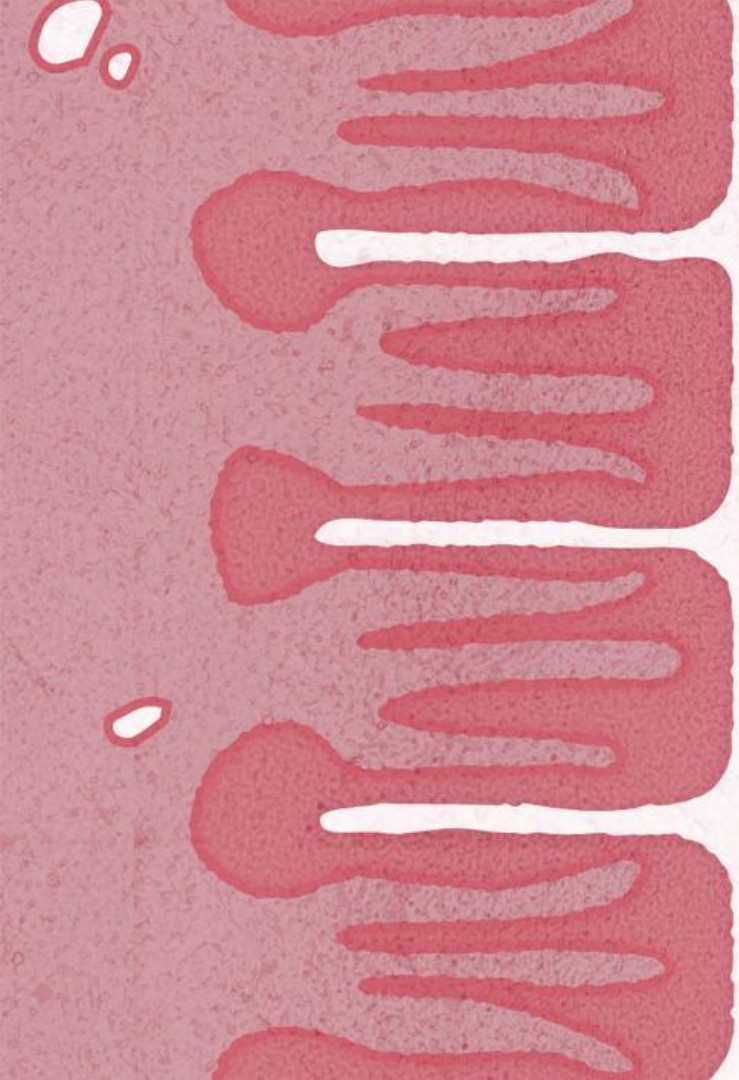
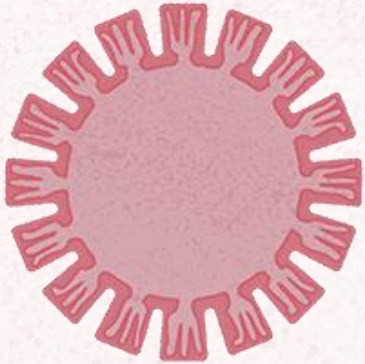


Zika Virus (ZIKV)

Eoghan Beary
Anthony Maguire
Ben Shanahan
Shane Morgan
Matthew Lyons

To Be Discussed..



01 Introduction, History and Preventative Measures

02 Modes of Transmission & Symptoms / Disorders

03 Structural Biology

04 Mechanism of Infection / Virology

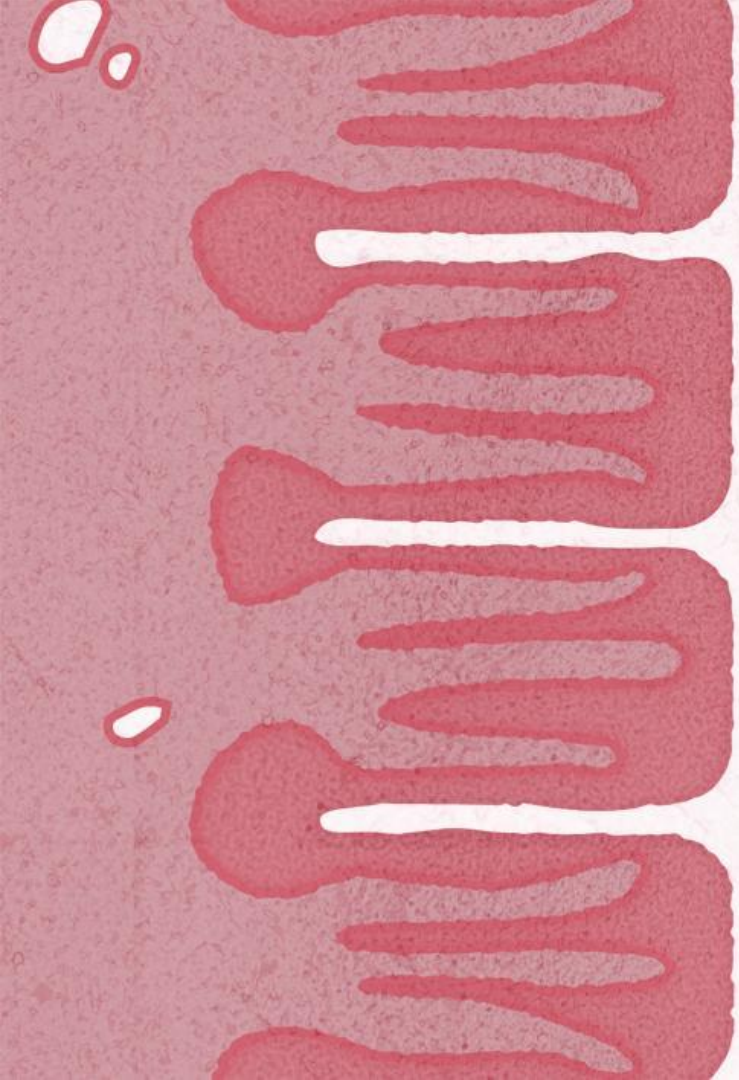
05 Diagnostic Testing

06 Vaccination & Drug Targets

07 Prevalence & Conclusion

Learning Objectives:

1. Become familiar with Zika virus, its history and preventative measures.
2. Understanding the modes of transmission of ZIKV, in addition to the symptoms and disorders following infection
3. Become familiar with the structural biology, encoded proteins of the virus, and the importance in understanding their function.
4. To provide an overview of how the zika virus infects host cells and its virology
5. Insights into the tools used for diagnostic testing and future technologies
6. To outline the current vaccine developments and how they target the zika virus.
7. Current situation and direction of the Zika virus.

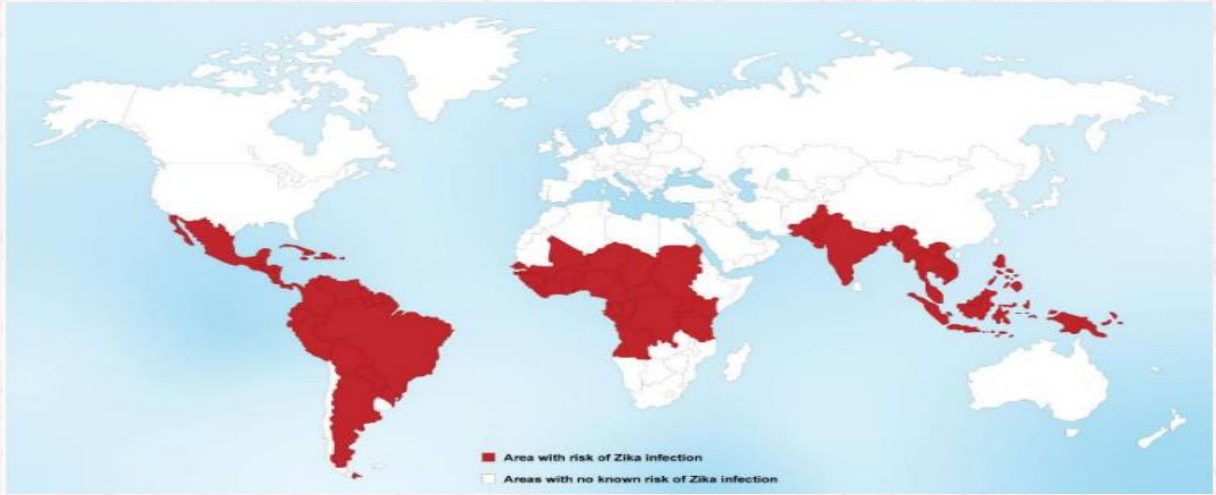


TOPIC 01

Introduction, History and Preventative Measures

Introduction – What is Zika Virus?

- Zika virus (ZIKV) is a member of the virus family *Flaviviridae*
- Spread by daytime-active *Aedes* mosquitoes



Global map of Zika virus infection

Question 01

Flaviviruses are responsible for which human disease?

a) Influenza

b) Dengue/Yellow Fever

c) Alzheimer's

d) Smallpox

ZIKV Timeline

1947

First discovered in Uganda

1952

First human case

2007

First serious outbreak – Island of Yap (western Pacific Ocean)

2015

Expansion into the Americas

2016

WHO declares Public Health Emergency of International Concern (PHEIC)

2018

More than 90 countries affected

2007 Yap Islands outbreak

First time Zika virus had been detected outside Africa and Asia

49 confirmed and 59 probable cases of Zika virus disease

Prior to this no outbreaks and only 14 cases of human ZIKV had been documented worldwide

2013-14 outbreaks in Oceania

In October 2013 reports surfaced from French polynesia of a ZIKV outbreak

Estimated to be 19,000 cases

ZIKV strain circulating in French Polynesia identical to Yap island strain

2015-2016 ZIKV epidemic

Early 2015

Zika virus spread from Brazil to other parts of South and North America

February 2016

PHEIC declared by WHO as evidence grew that ZIKV can cause birth defects and neurological problems

November 2016

WHO announced the end of the Zika epidemic

711,381

Total ZIKV cases 2015-16 (Including 15 in Ireland)

Total ZIKV deaths 2015-2016: **18**

Question 02

In what year did WHO declare a PHEIC due to ZIKV?

a) 2010

b) 2014

c) 2016

d) 2018

Preventative Measures

1

Mosquito population

Reducing the mosquito population by chemical methods

2

Mosquito Breeding sites

Eliminating or reducing mosquito breeding sites such as standing water reservoirs

3

Government penalties

Introducing penalties against standing water reservoirs

4

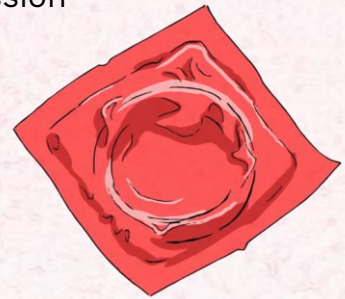
Educating people

Who are at risk about the benefits of mosquito netting and topical insecticides

5

Prevent sexual transmission

Using condoms to prevent sexual transmission



GM Aedes Aegypti Mosquito

- Brazils National Biosafety committee approved them in response to the epidemic
- Male GM mosquitoes mate with female to transmit gene causing offspring to die
- Environmental Protection Agency in May of 2020, approved the release of 750 million GM mosquitos into the Florida Keys throughout 2021 and 2022.
- Oxitec study 90% reduction in mosquito population
- ***Ref:*** ***GM Aedes Aegypti Mosquito***

TOPIC 02

Modes of Transmission & Symptoms/Disorders

Modes of Transmission

01 Mosquito-Borne Transmission

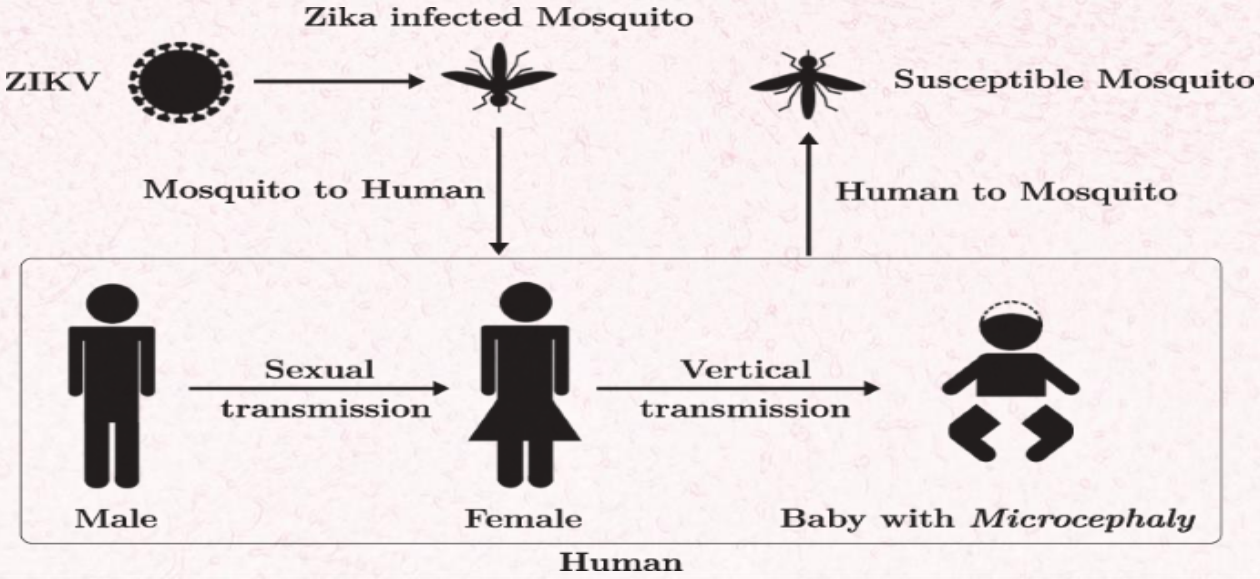
- Transmission Cycle
- Mosquito Transmission in Humans

02 Sexual Transmission

- Studies on Males and Females
- Sexual Transmission Framework

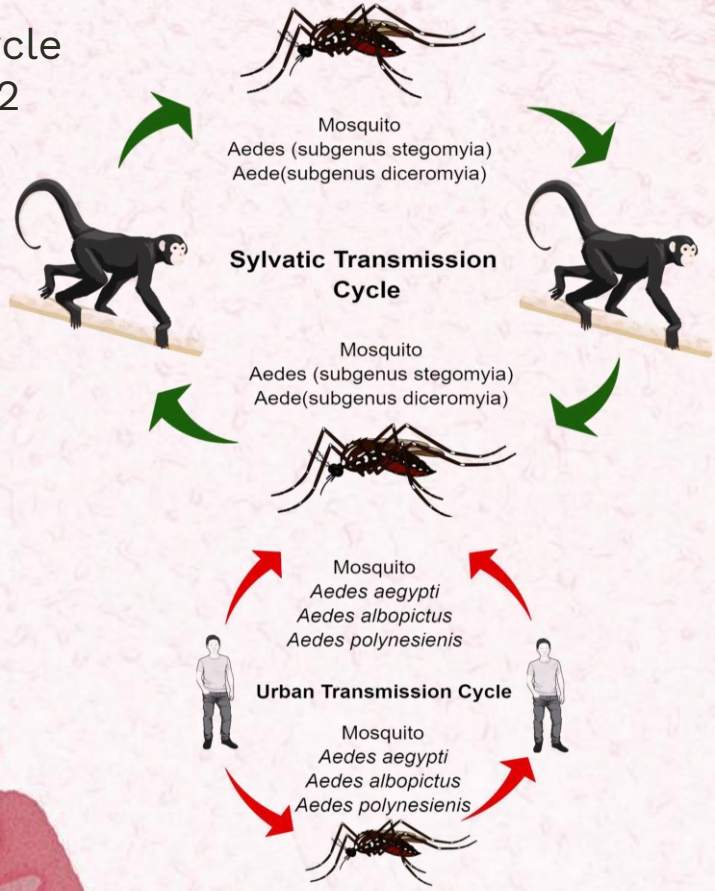
03 Vertical Transmission

- Routes of Transmission
- Congenital/Intrauterine
- Perinatal Transmission



Mosquito-Borne Transmission

Transmission Cycle Composed of 2 Components:

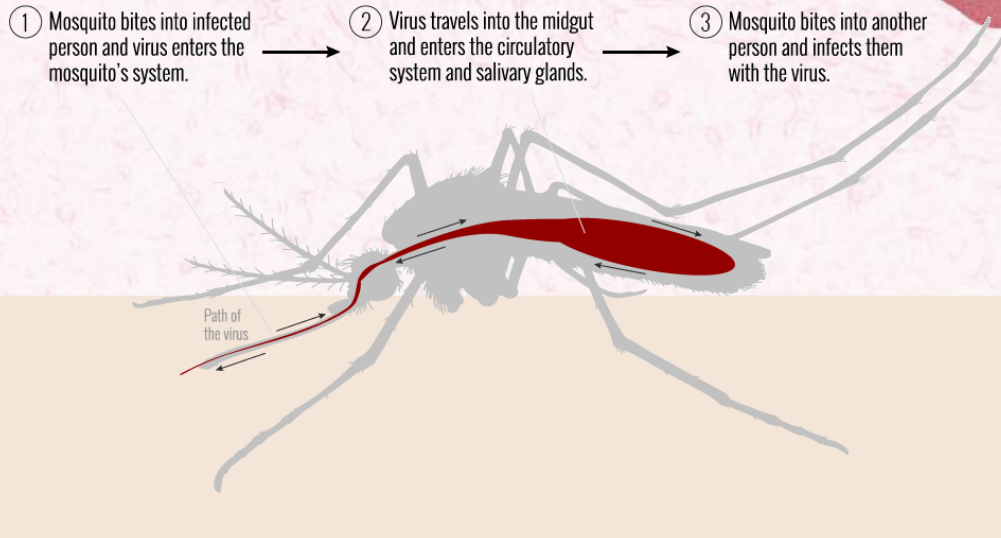


- In Africa, ZIKV circulates in a sylvatic transmission cycle with the inclusion of human exposure.
- ***Aedes aegypti*** is the main mosquito vector associated with ZIKV transmission:
- High vectorial capacity due to close association with human habitation

Mosquito-Borne Transmission

- ***Aedes*** mosquito transmits numerous arboviruses such as Zika, dengue, chikungunya and yellow fever virus.
- Predominant ZIKV vector transmitter is the ***Ae. aegypti*** with other ***Aedes*** species capable for ZIKV transmission such as; ***Ae. africanus***, ***Ae. albopictus*** (Contribution to ZIKV transmission within parts of Europe and Mediterranean areas), and ***Ae. polynesiensis*** (Transmission in French Polynesia).

Mosquito Transmission in Humans:



Question 03

Which ***Aedes*** species contributed ZIKV transmission within parts of Europe and Mediterranean areas?

a) Aedes africanus

b) Aedes albopictus

c) Aedes polynesiensis

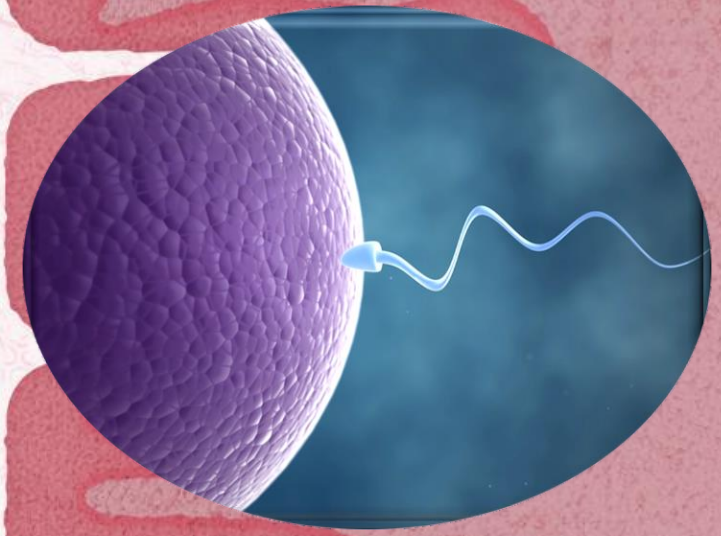
d) Aedes aegypti

Sexual Transmission

- Sexual Transmission of ZIKV was first reported in 2008, Colorado, USA.
- Vast majority of reports of ZIKV transmission has occurred from symptomatic male-to-female transmission with fewer cases involving symptomatic female-to-male and asymptomatic male-to-female transmissions documented.

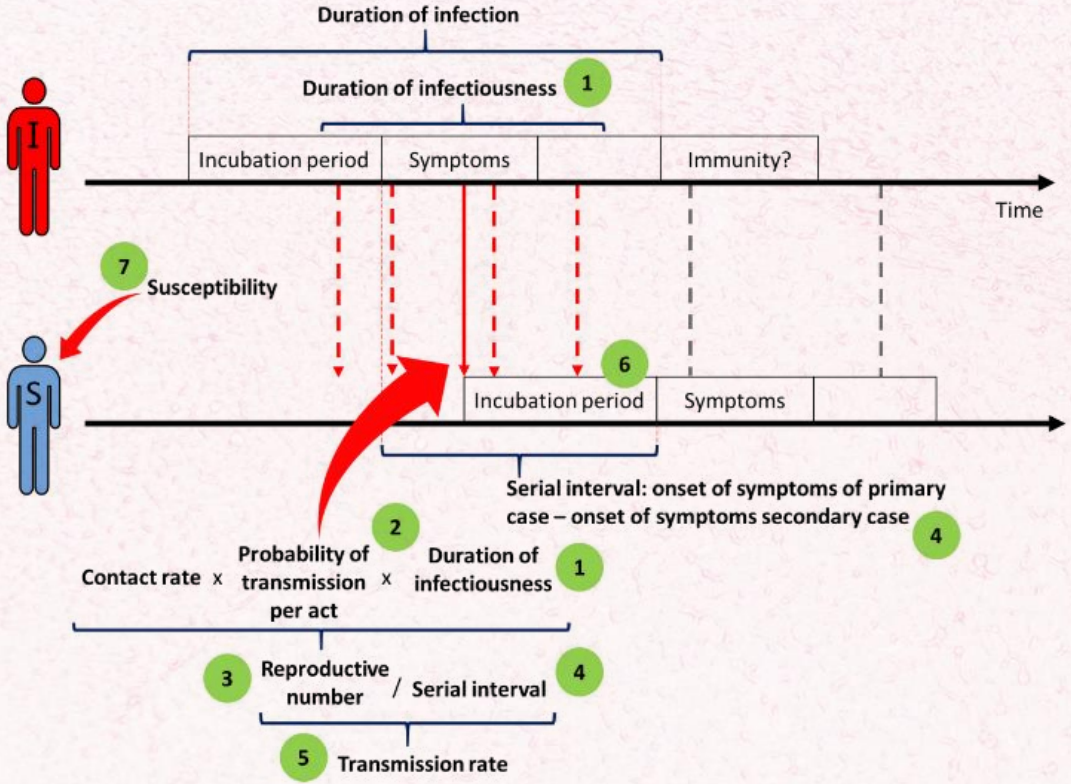
- Presence of ZIKV is semen has been assessed in 2 ways:
  - ZIKV RNA detectable by RT-PCR
  - Demonstration of Virus by passage amplification in culture

- Study involving female participants in Puerto Rico has indicated shedding of ZIKV RNA in vaginal secretions has been deemed uncommon and limited in duration (usually <14 days).



The longest published time point of ZIKV RNA detected in semen (after onset of illness) has been reported to be as long as 188 days.

Sexual Transmission Framework (STF)



- Development of the STF allowed for quantification of the potential sexual transmission of ZIKV.
- STF aimed to determine dynamics of ZIKV sexual transmission which brought in the realisation of ZIKV's epidemic potential.
- ***Ref:*** ***WHO STF Development 2017***

Question 04

What was the longest published time point of ZIKV RNA detected in semen (after onset of illness) reported to be?

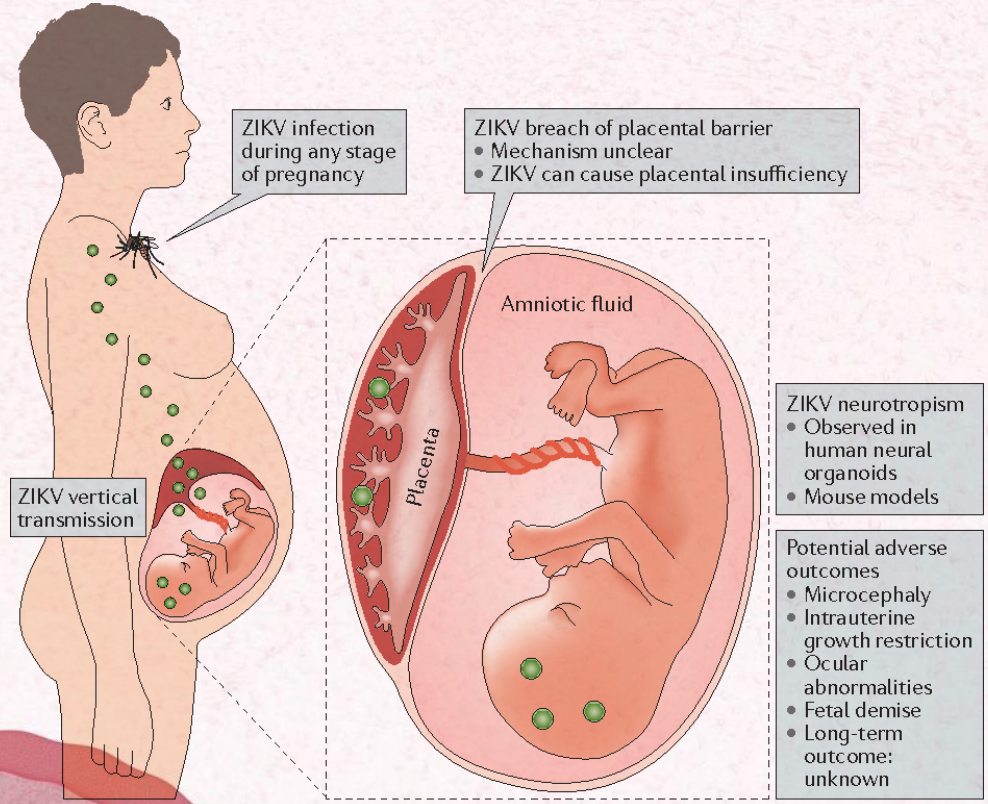
a) 120 days

b) 172 days

c) 155 days

d) 188 days

Vertical Transmission



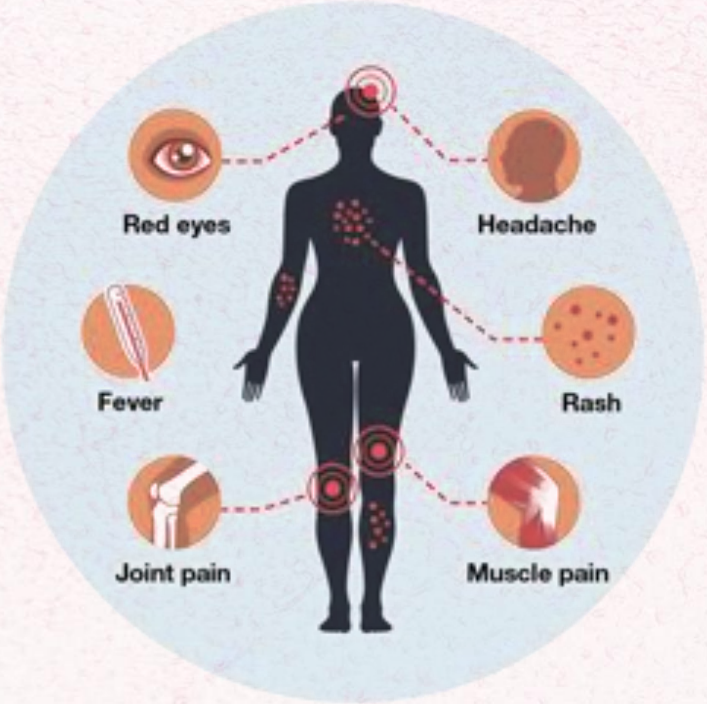
- Pregnant women can become infected with ZIKV in which maternal-foetal ZIKV transmission can occur throughout pregnancy.

- The main routes of vertical transmission for ZIKV would be placental (transplacental) and across the female reproductive tract during childbirth.

➢ **Congenital/Intrauterine** transmission occurs when a women becomes infected with ZIKV during pregnancy (before delivery)

➢ **Perinatal** transmission occurs when a women becomes infected within 2 weeks of delivery, where ZIKV is passed to the baby at the time of delivery

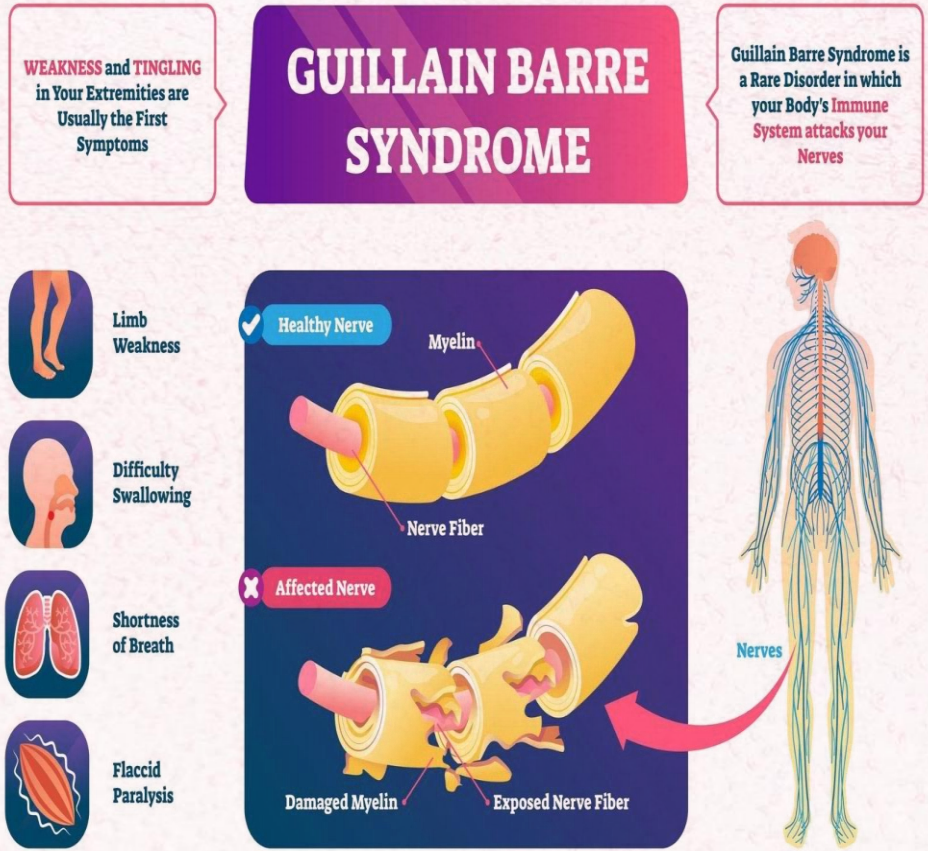
Symptoms



- Majority of people infected with ZIKV display little to no symptoms, and if so, the symptoms are usually mild, lasting between 2-7 days.

Nothing To Be Scared Of?

Guillain-Barré Syndrome (GBS)



- An immune-mediated flaccid paralysis.
- In 1916, George Guillain, Jean Alexandre Barré, and André Strohl described an acute flaccid paralysis characterized by high protein levels in the cerebrospinal fluid without increase in inflammatory cells.
- Paralysis is usually transient with most people recovering between 6-12 months.
- Current treatments for GBS:
  - Plasmapheresis
  - Immunoglobulin Therapy

Question 05

What is the normal recovery period of paralysis for a person affected with Guillain-Barré Syndrome?

a) 6-12 months

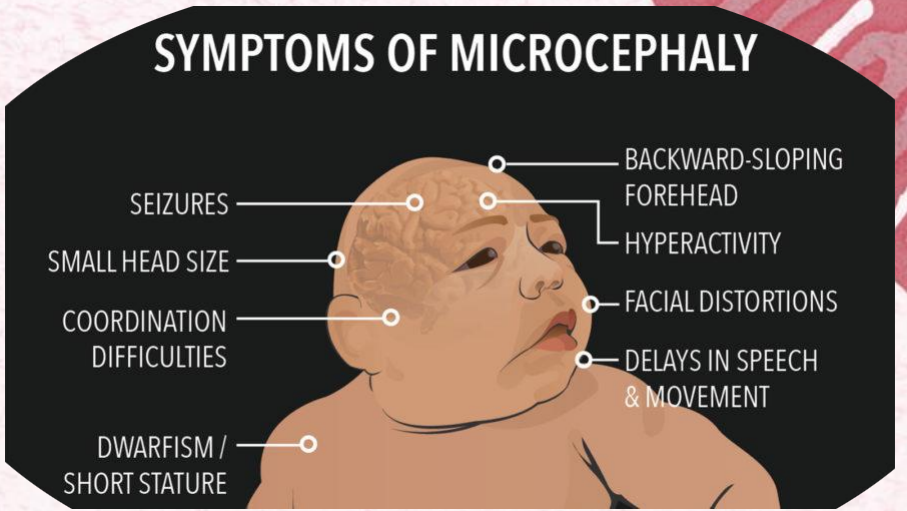
b) 12-24 months

c) 24-36 months

d) 0-6 months

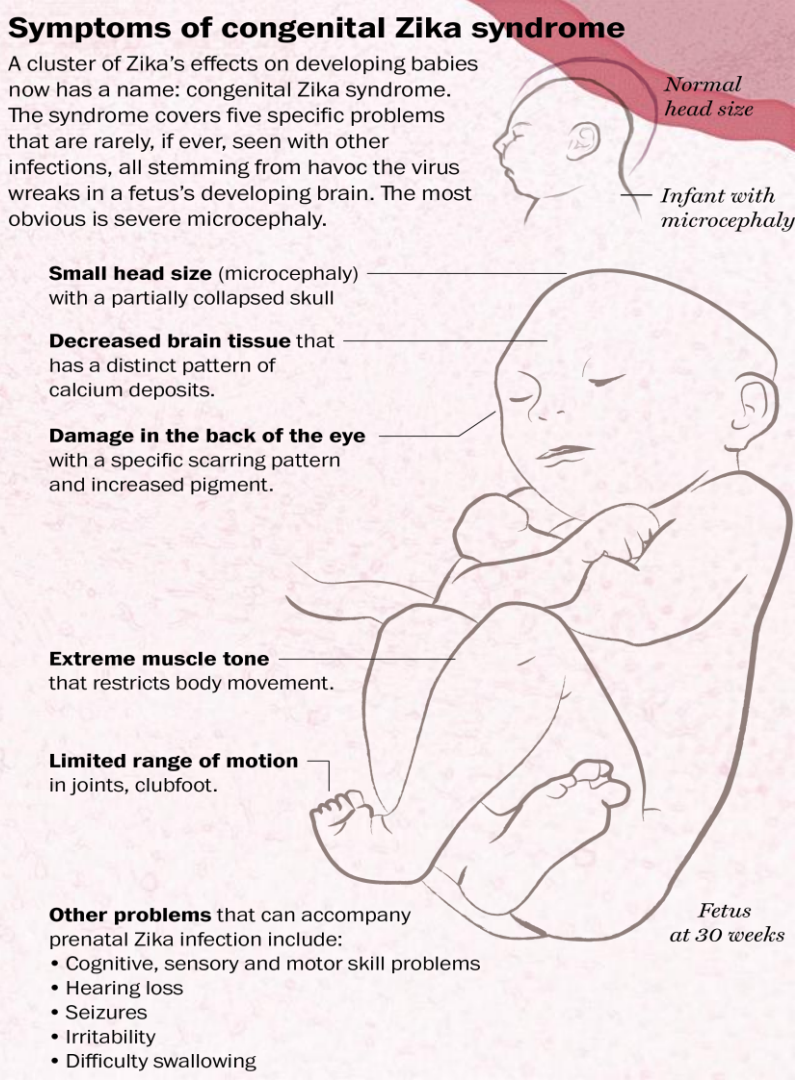
Microcephaly

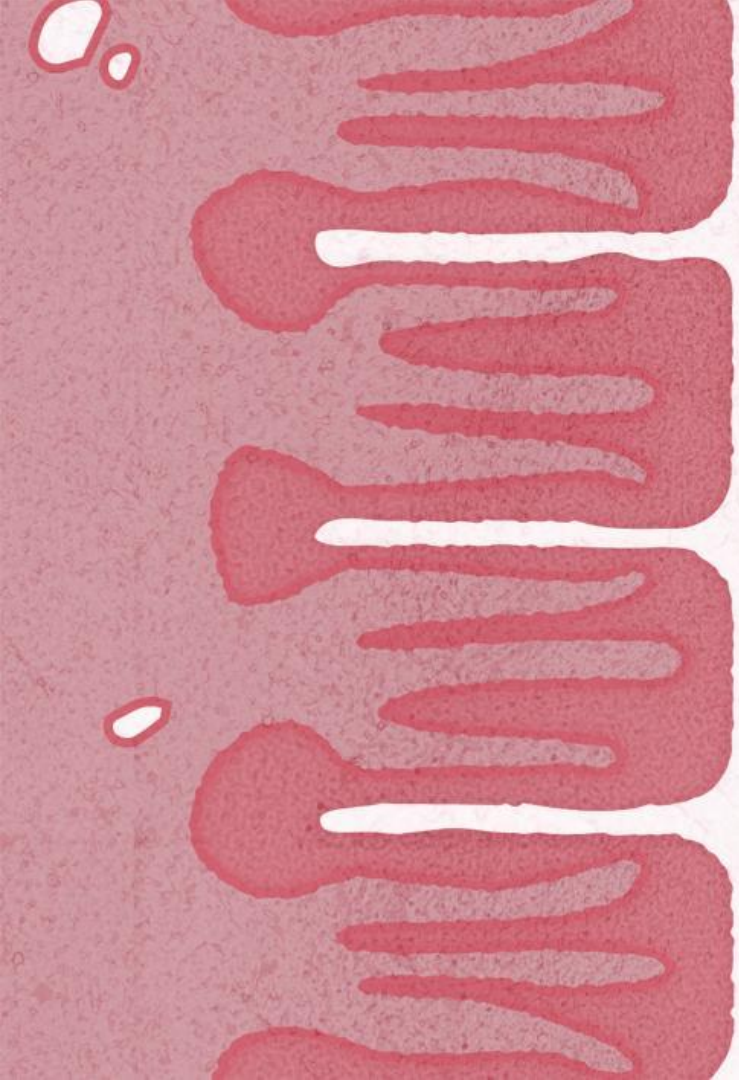
- After 2015's ZIKV outbreak in Brazil, an incremental increase of microcephaly cases in newborns occurred.
- Caused reduction of head circumference by roughly 2 standard deviations.
- 2016, the first case of ZIKV detection in brain tissue of a foetus with microcephaly in Rio Grande de Norte State, Brazil



Congenital Zika Syndrome

- Congenital Zika Syndrome includes the following birth defects:
- ➢ Severe microcephaly, inclusive of a partially collapsed skull
- ➢ Decreased brain tissue with a specific pattern of brain damage, including subcortical calcifications
- ➢ Damage to the back of the eye, with macular scarring
- ➢ Congenital contractures, such as clubfoot or arthrogryposis
- ➢ Hypertonia restricting body movement soon after birth





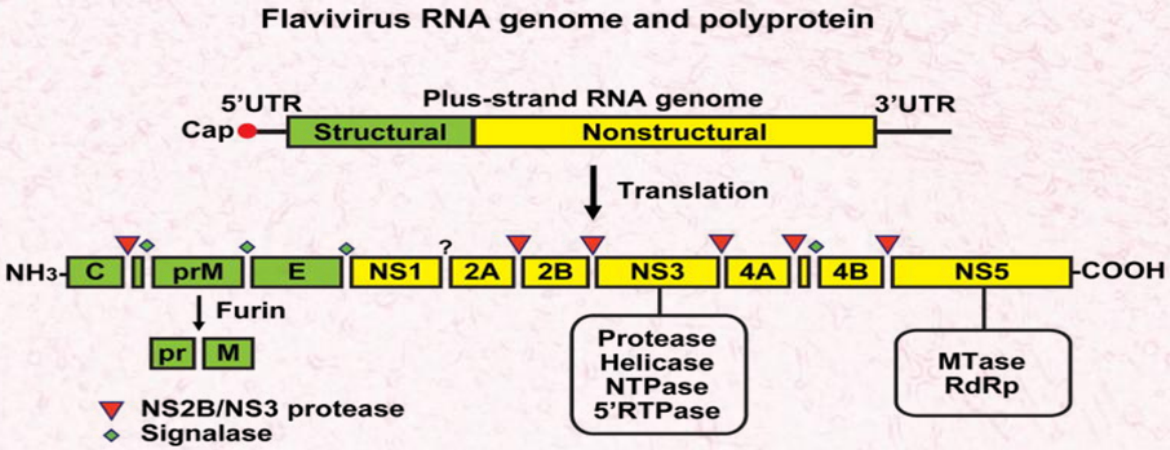
TOPIC 03

Structural Biology

Points for discussion:

- Genome
- Structural and non-structural proteins
- Importance in the understanding of particular proteins

Flavivirus Genome



Common genome across all flaviviruses such as:

- West Nile virus (WNV)
- Dengue virus (DENV)
- Yellow Fever virus (YVF)
- Japanese encephalitis virus (JEV)

Translation of the genome is facilitated by host cell machinery.
This produces a polyprotein comprising of:

- Structural proteins
- Non-structural proteins

Question 06

Which of the following viruses does not share the common genome across all Flaviviruses?

a) Rubella virus

b) West Nile virus

c) Dengue virus

d) Japanese encephalitis virus

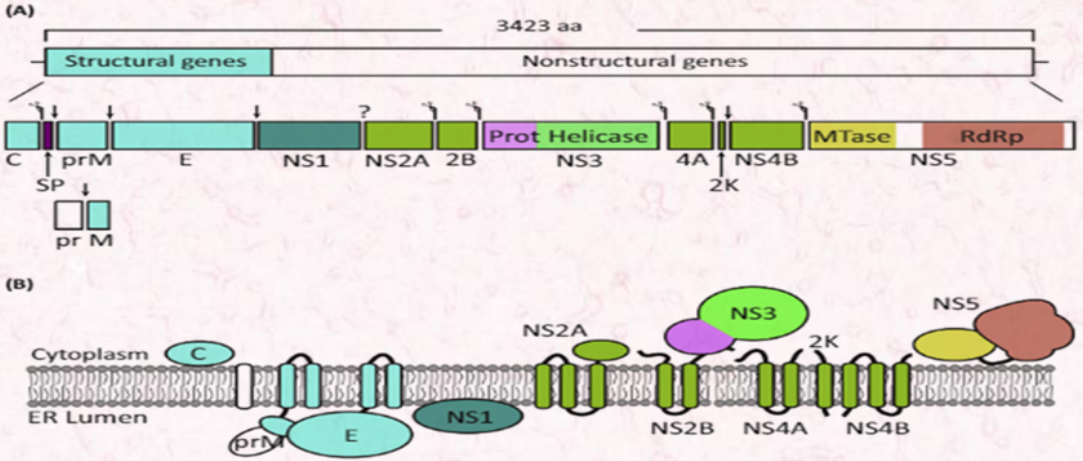
Zika Virus Open Reading Frame (ORF)

The Zika virus genome contains an ORF that codes for all its structural and non-structural components.
The encoded structural components contain the:

- Capsid (C) and membrane (M) proteins.

The non-structural portion includes the:

- Envelope (E) and 7 other proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5).



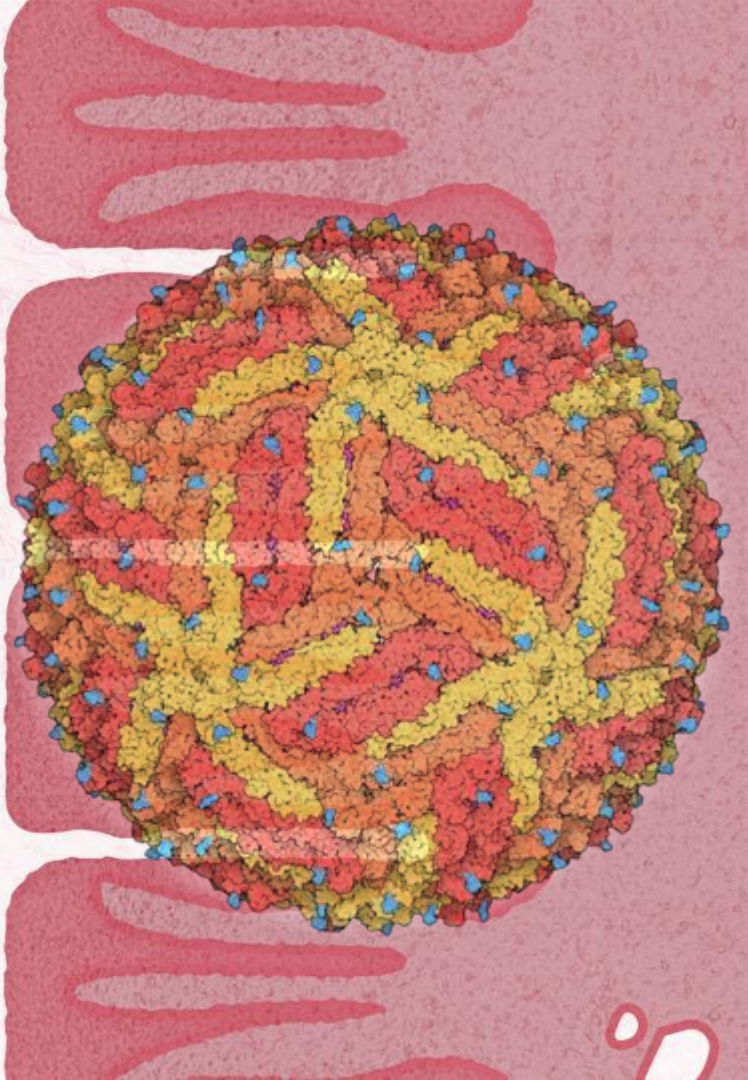
Structure – E Protein

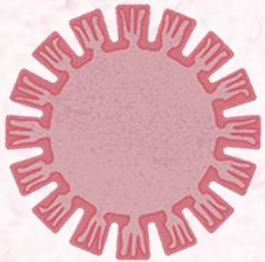
Zika virus comprises an icosahedral shell.
Within this shell contains 180 copies of each of the E and M proteins.

E proteins are the major component involved in:

- Receptor binding
- Membrane fusion
- Host immune recognition

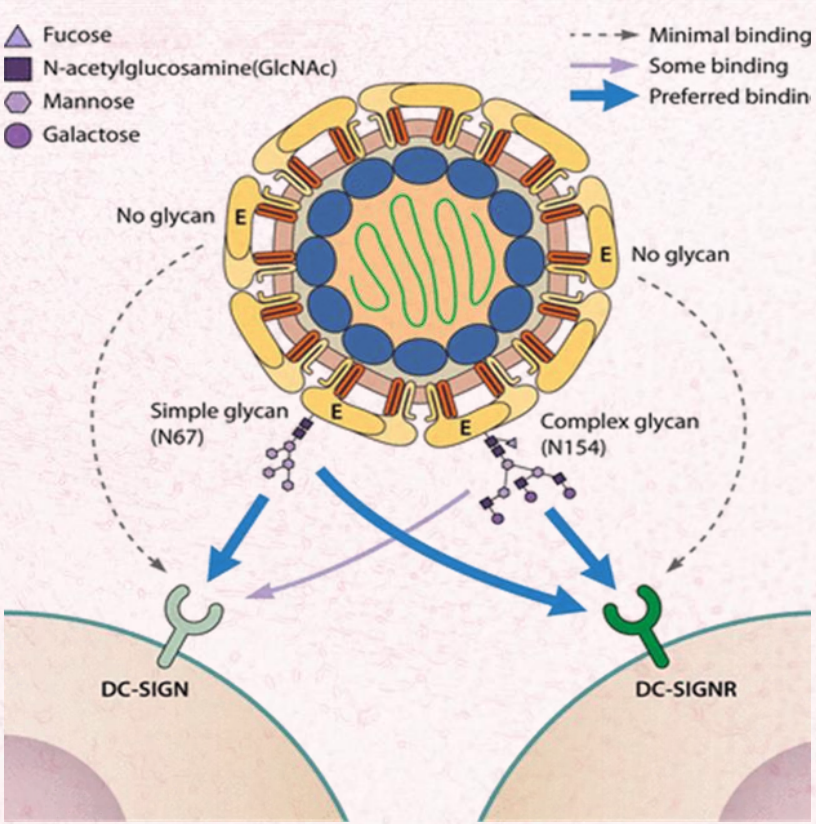
Here we can see the envelope protein structure. The yellow and red colours represent the envelope proteins with blue colouration representing glycosylation.





Glycosylated E Protein

- The image shows glycosylated E proteins on the viral package interacting with DC-SIGN and DC-SIGNR on cell surfaces.
- These proteins are expressed on microvascular endothelial cells such as the liver, lymph nodes, and placental villi.
- E glycosylated ZIKV strains use DC-SIGN/R as attachment factors, leading to enhanced infection compared to nonglycosylated strains



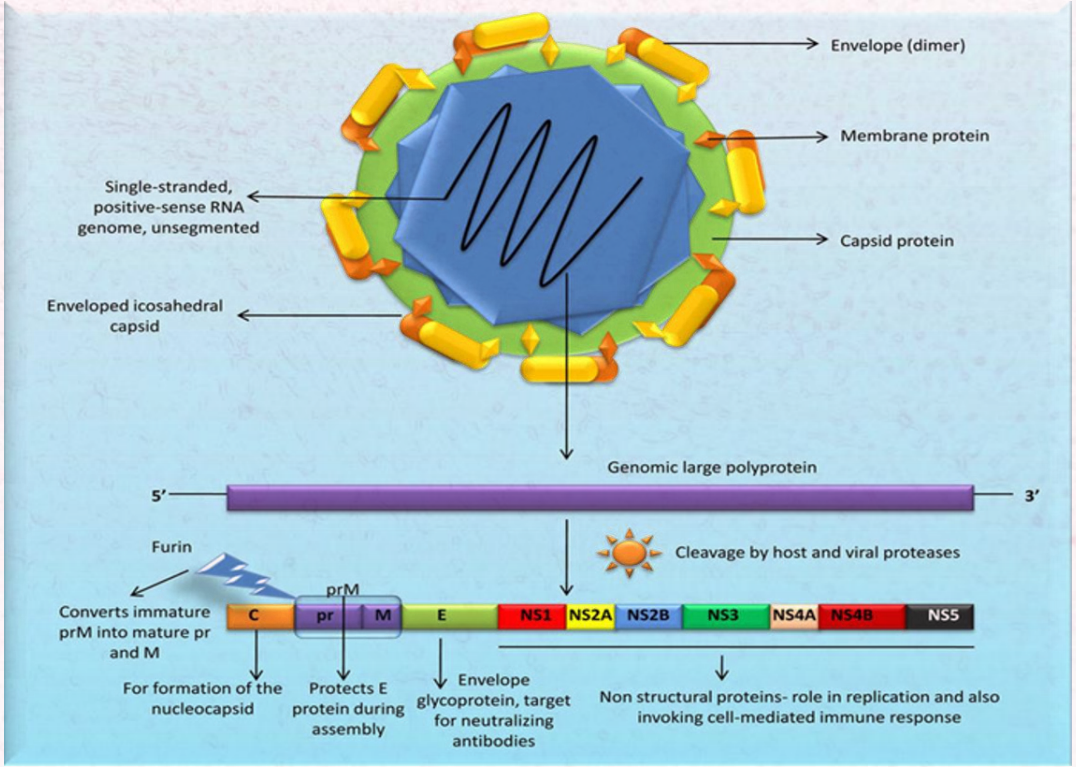
Structure – C Protein

•The C protein interacts with viral genomic RNA.

•This forms a nucleocapsid.

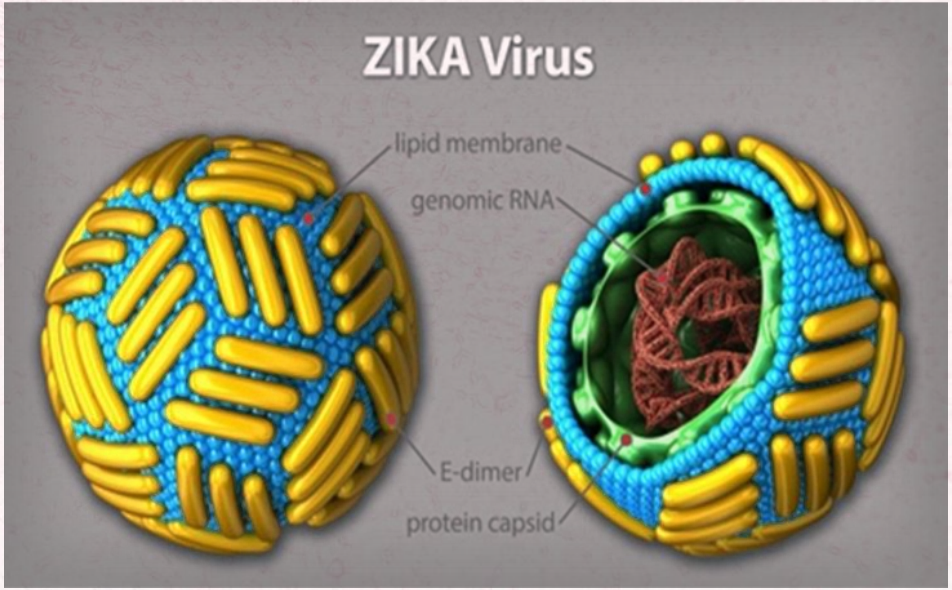
•The capsid protein comprises an internal hydrophobic sequence that mediates membrane association.

•The C- terminal region of this sequence is involved in RNA association.



Structure - M Protein

- The M protein is hidden under the E-layer
- Precursor to M proteins are prM proteins.
- These immature particles transit through low pH environments in the Golgi compartment.
- Plays an important role in the assembly of mature virions through cleavage of prM into M protein.



- Most vaccines targeting ZIKV include prM as the immunogen

Non-Structural Proteins

Proteins name	Symbol	Size (aa)	Location in cell	Function in cell
NS1 protein	NS1	384	Cytoplasm	Emission, virulence, and replication
NS2 protein	NS2A	226	Cytoplasm	Viral transcription and assembly
NS2 protein	NS2B	130	Cytoplasm	NS3 cofactor for serine protease function, polyprotein cleavage
NS3 protein	NS3	617	Cytoplasm	Unwinding of structured protein template region and processing of viral polyprotein via serine protease, helicase and triphosphatase activity
NS4 protein	NS4A	127	Cytoplasm	Viral replication
NS4 protein	NS4B	252	Cytoplasm	Viral replication complex
NS5 protein	NS5	902	Cytoplasm	RNA replication via RNA dependent RNA polymerase and RNA capping

Ref: ***Non-Structural Proteins List***

Question 07

Which Zika Virus protein interacts with CD-SIGNR and DC-SIGNR proteins on endothelial cell surfaces?

a) C-Protein

b) M-Protein

c) E-Protein

d) NS-Protein

Proteins of Interest

Protein	Why
• Envelope (E)	• Elicits strong humoral immune response, causing the production of immunoglobulin antibodies (IgM).
• Membrane (M)	• Cleavage of prM to M by cellular serine proteases results in conformational protein structure changes.
• Capsid (C)	• Dimerization of C- is induced by interaction with DNA or RNA.
• NS1	• Involved in the viral replication complex. When glycosylated, binds to uninfected cells.
• NS3 and NS2B-NS3	• NS3 helicase works with NS5 polymerase unwind template regions of RNA during viral RNA synthesis. • NS2B-NS3 serine protease is essential in processing, assembly, and replication of the virus
• NS5	• NS5 is the largest protein encoded in the flavivirus genome and is the most conserved one among viral proteins. This protein plays a crucial role in flavivirus replication.

TOPIC 04

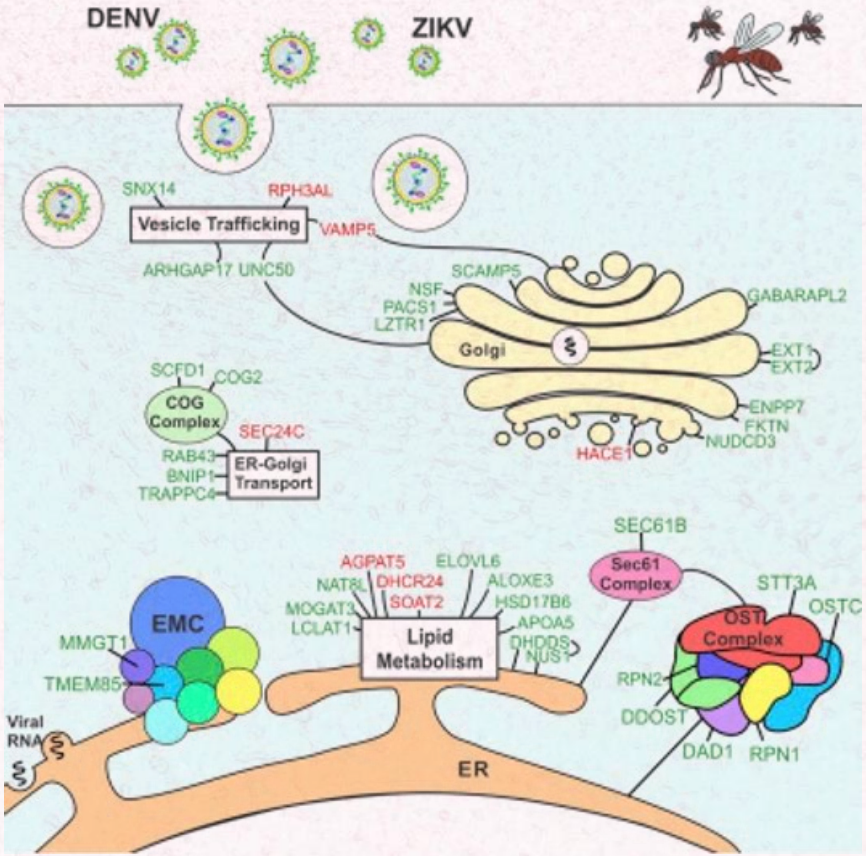
Mechanism of Infection / Virology

Mechanism of Infection

- ZIKV infections occur following an interaction between the surface cell receptors in the host cell and the viral surface glycoproteins.

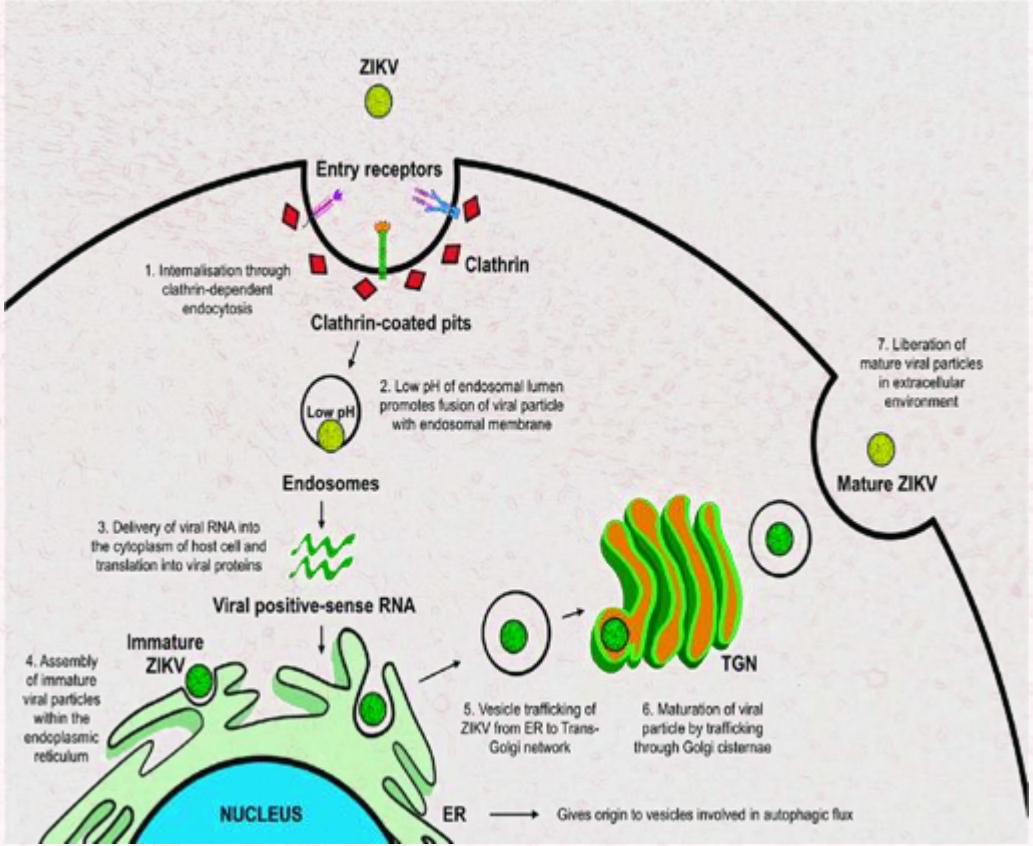
- Multiple families of proteins have been suggested as possible entry receptors including AXL family receptor tyrosine kinases.

- ***It is a BSL-2 (Biosafety Level 2) Pathogen.***

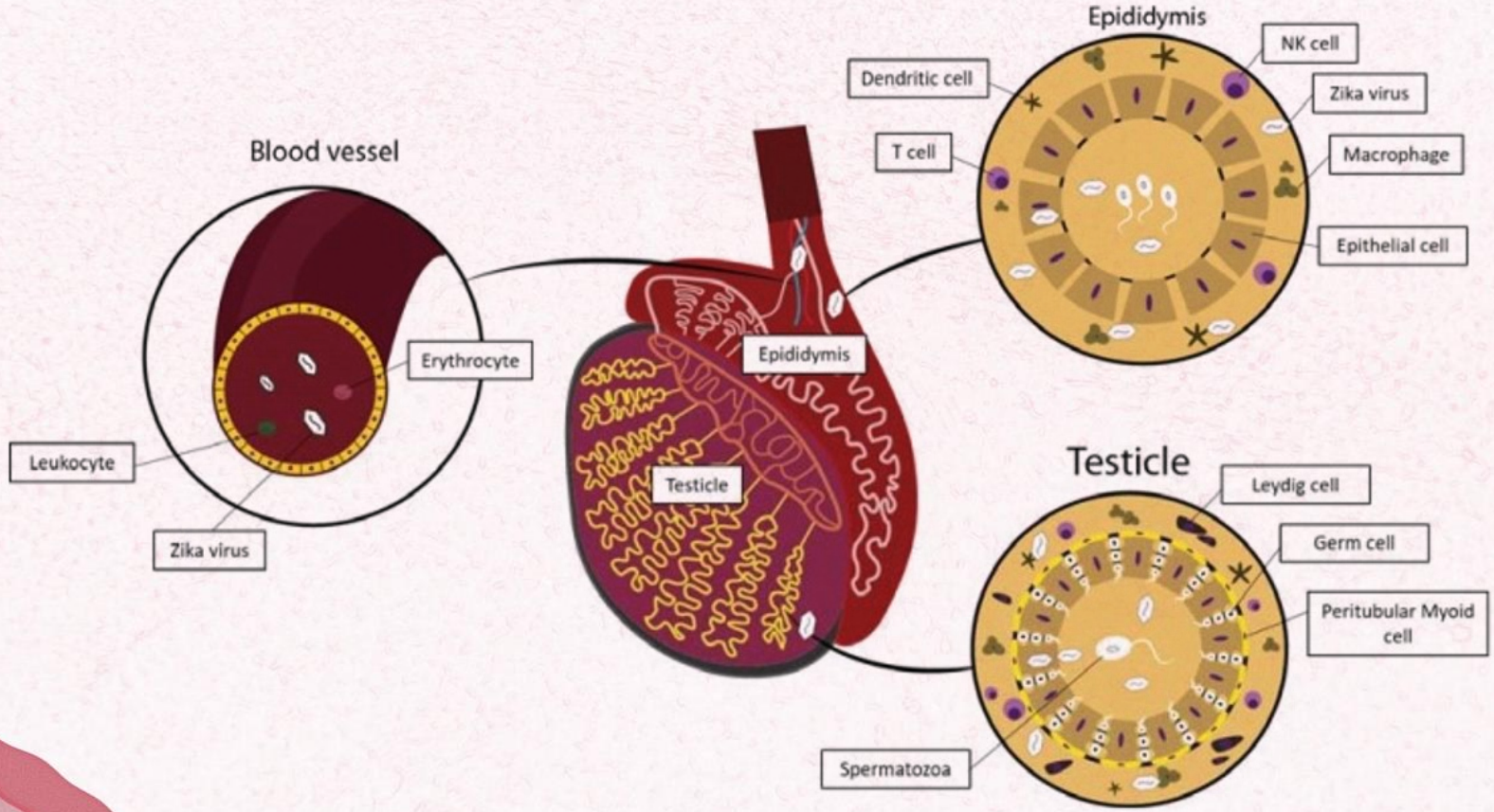


Mechanism of Infection

- Low pH of endosomal lumen allows fusion of viral particle to endosomal membrane.
- The zika virus assembles immature viral particles within the ***endoplasmic reticulum.***
- Clathrin is a protein that plays a major role in the formation of coated vesicles.



Zika and the Human Male Reproductive Tract



Question 08

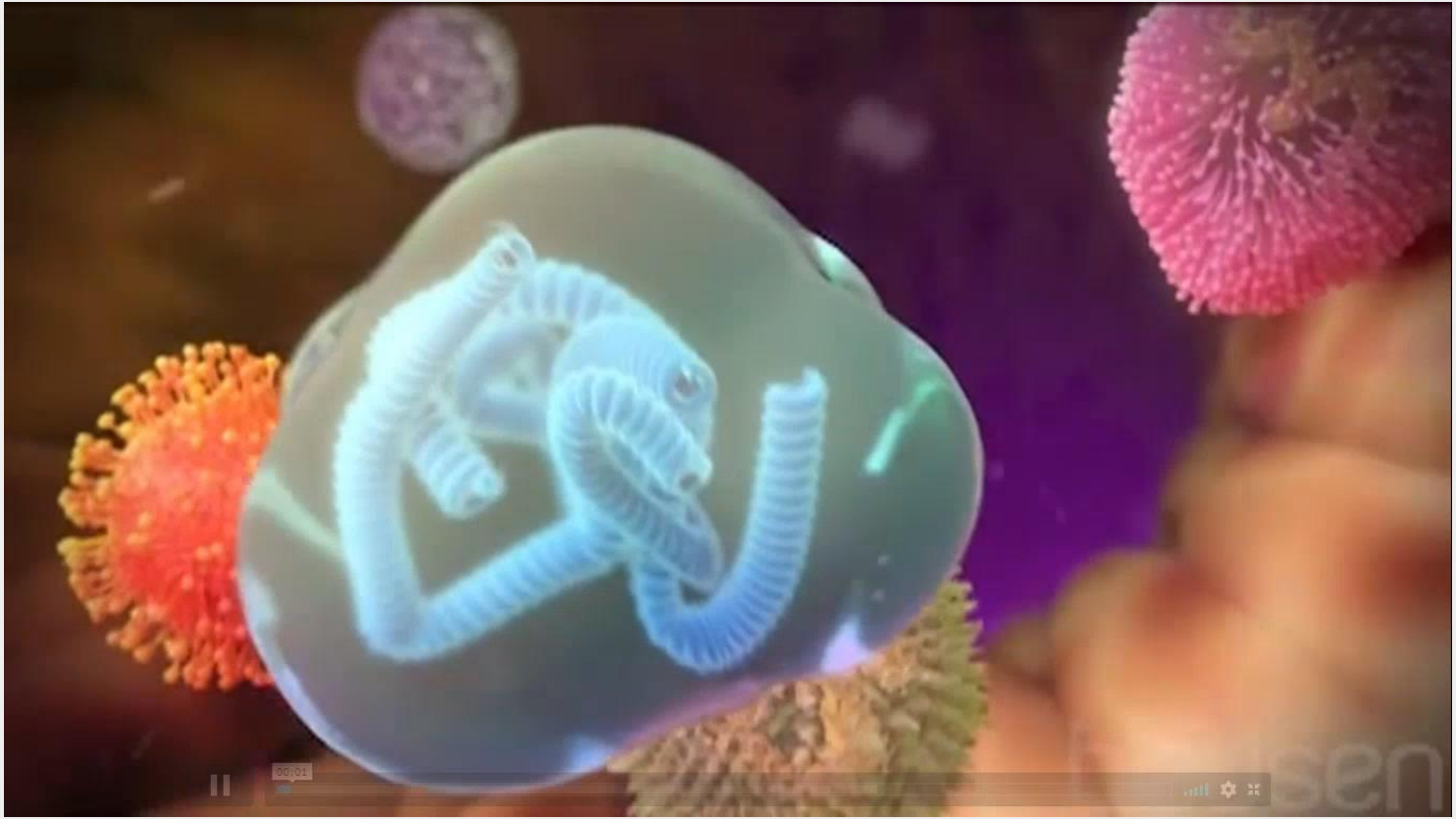
Which protein plays a role in the formation of coated vesicles?

a) Clathrin

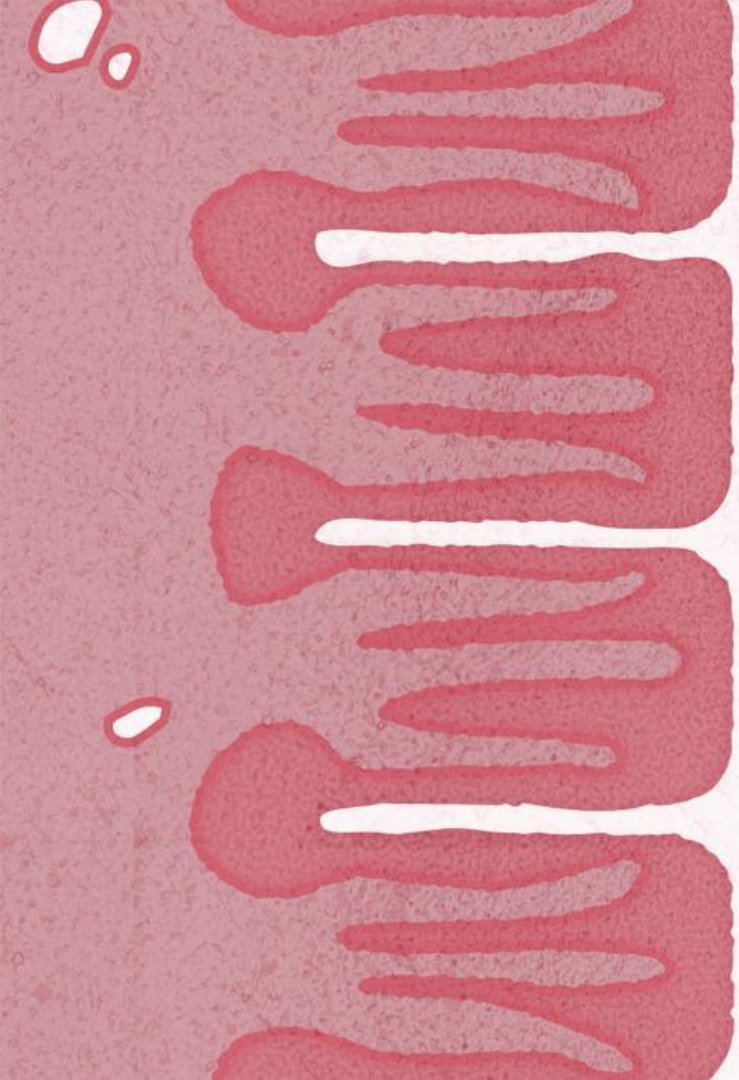
b) Keratin

c) Myosin

d) None of the above



Ref: ZIKV Animation

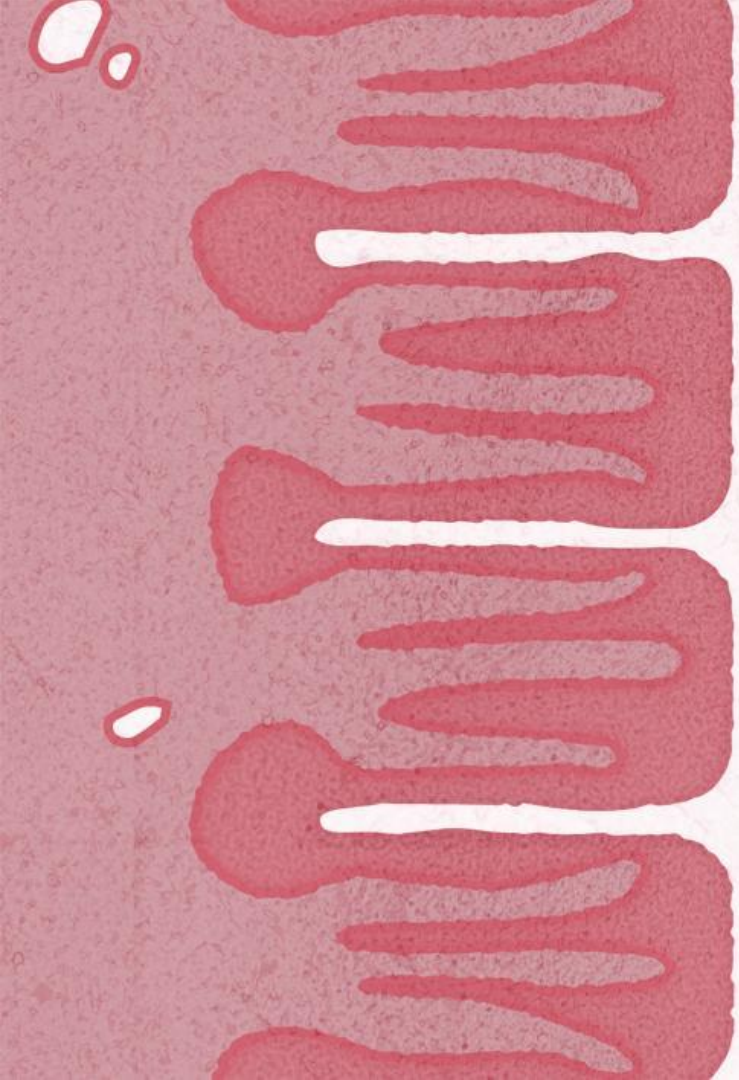


Locations of Infection

- The zika virus appears in cells and tissues that have not been previously seen in humans.

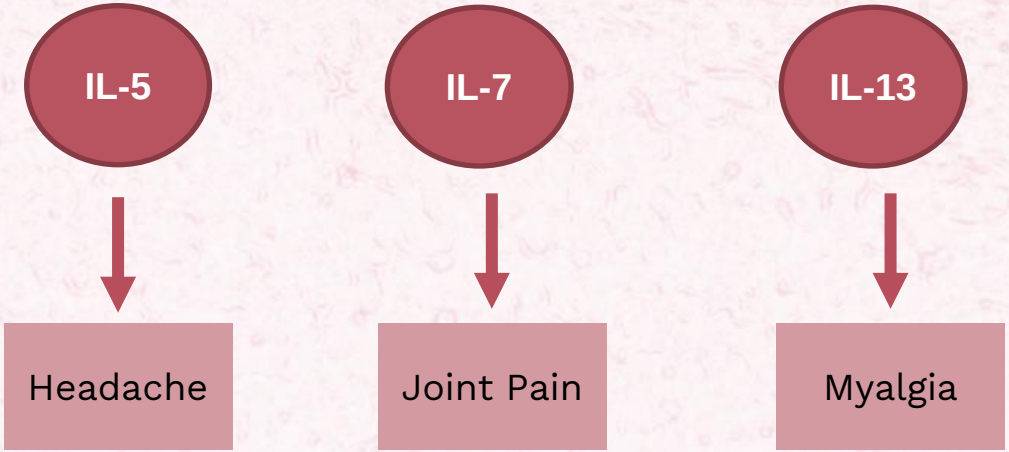
- Study detected infectious ZIKV particles in the urine and saliva of patients during the acute phase of infection.

- ZIKV particles can remain in the plasma for up to **two weeks** and in the urine for up to **39 days.**



Zika Virus Cytokines

- Cytokines are proteins, peptides or glycoproteins secreted by specific cells of the immune system.



Question 09

For how long can the ZIKV particles remain detectable in the plasma?

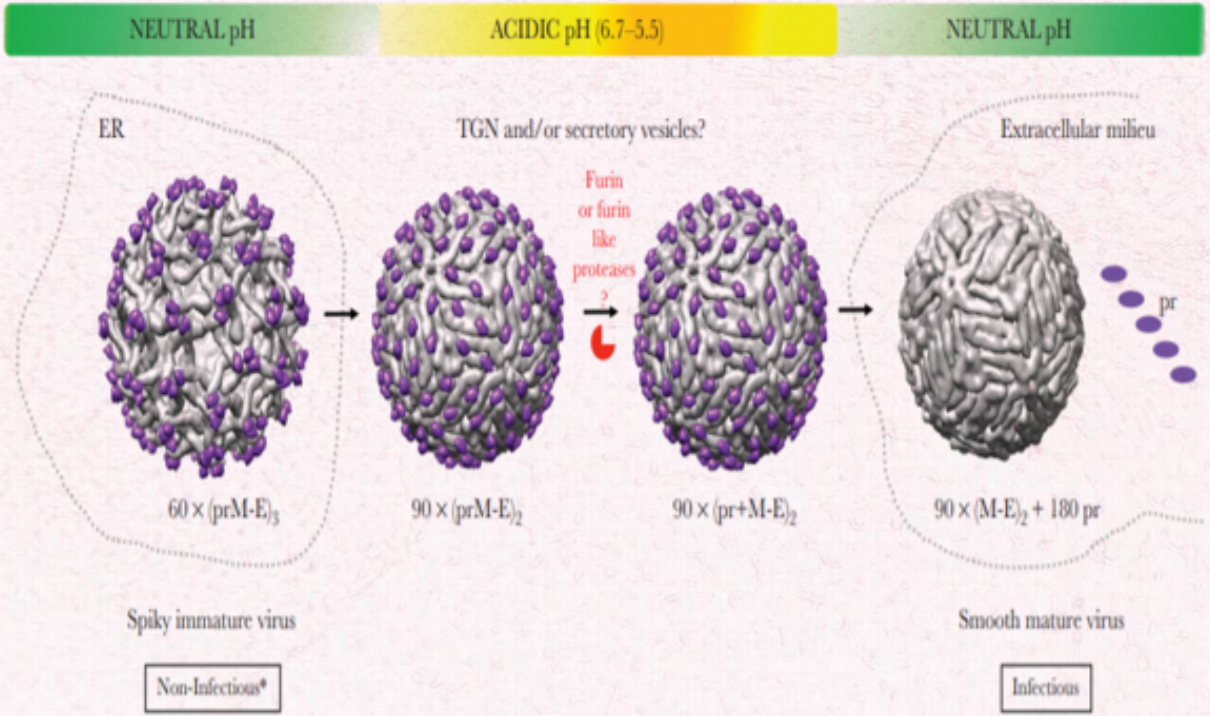
a) 2 weeks

b) A day

c) A month

d) None of the above

Maturation Pathway



- The immature zika virus has 3 prM-E heterodimers.
- These come together and project outward from the membrane with the distal fusion loop of E protein at its apex.
- The precursor domain of the prM is positioned at the top of the spike.

Question 10

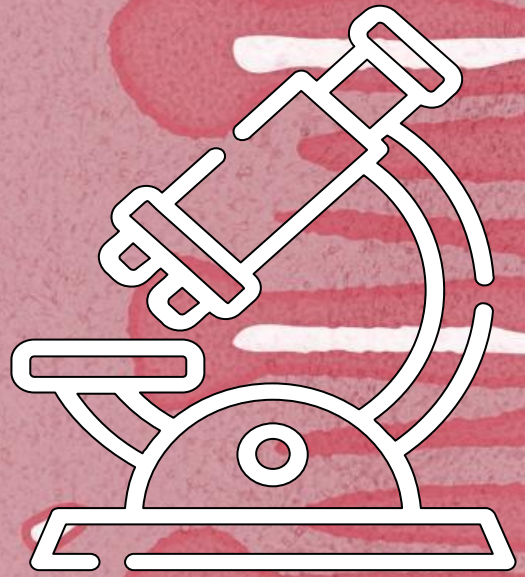
How many prM-E heterodimers does the immature zika virus have?

a) 3

b) 300

c) 3000

d) None of the above

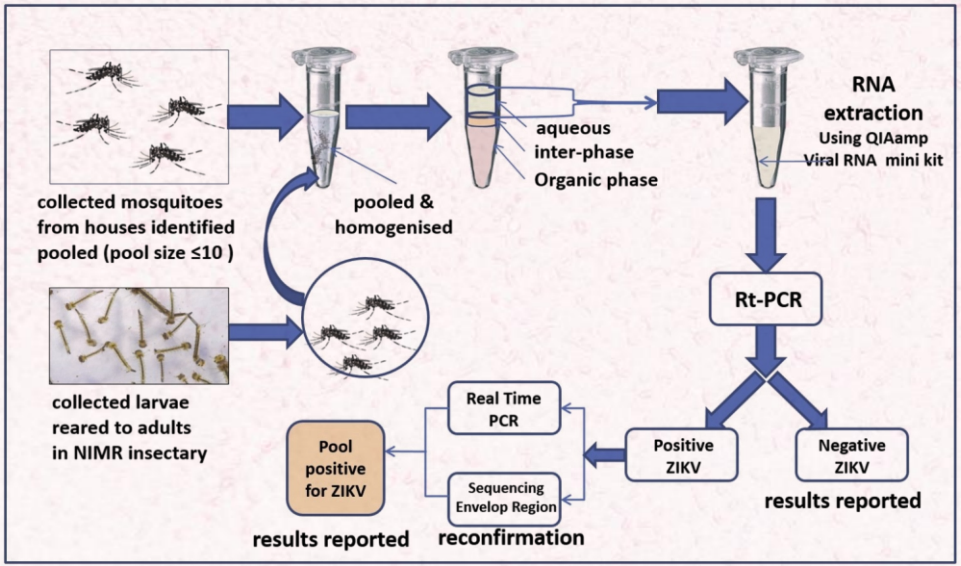


TOPIC 05

Diagnostic Testing

01 Eurofins ZIKV Real Time RT-PCR

- RT-PCR is routinely used to identify ZIKV RNA or ZIKV serology.
- ZIKV RT-PCR is intended for qualitative detection of RNA from ZIKV in human plasma, serum, or urine.



- Diagnosis of ZIKV infection is made by recognition of viral nucleic acid in the sample.
- The efficacy of RT-PCR is clear if undertaken in the first week after commencement of clinical symptoms dues to viraemia being brief.

- FDA issued an Emergency Use Authorization (EUA) for this product.

02 ZIKV Detect 2.0 IgM-capture ELISA

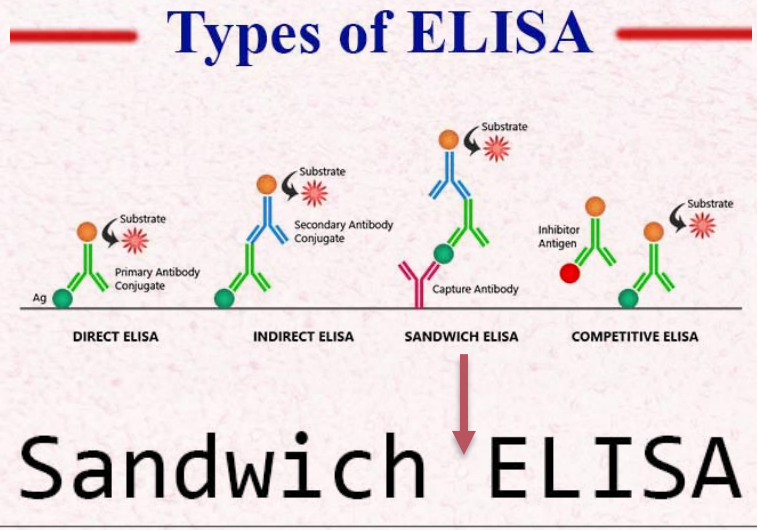
ZIKV Detect 2.0 IgM Capture ELISA Kit

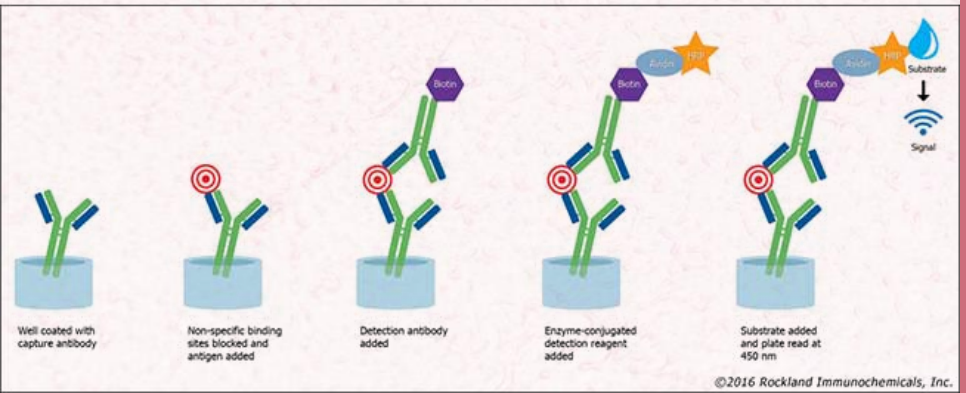
- Intended for qualitative detection of ZIKV IgM antibodies in human serum.
- A sandwich-type ELISA.

- Similarly to other arbo-flaviviruses, diminishment of ZIKV by the presence of active viruses in the blood results In IgM antibodies produced that can be detectable for several months.
- Detection of ZIKV-specific IgM antibodies by IgM-capture ELISA may be used for diagnosis of ZIKV infection 6 or more days after onset of clinical symptoms.

- FDA Approval on May 23rd 2019

ZIKV Detect 2.0 IgM Capture ELISA





Test Principle

- Polyclonal capture antibodies precoated on polystyrene microtiter wells.
- + Control, - Control & Unknown TS added to ELISA plate.
- After Incubation & Washing, RTU ZIKV antigen, CCA, and NCA added to each well followed by addition of RTU 2ndary antibody solution.
- Enzyme conjugation solution added to each well.
- Incubation by TBM substrate & Acidic Stop Solution Added.

Presence of IgM Antibodies

- Degree of enzymatic turnover by ABS Measurement @ 450nm.
- IgM antibodies targeting ZIKV present = Complex is formed consisting of IgM, antigen, 2ndary antibody and conjugate.
- No presence = antigen, antibody & conjugate washed away.

Question 11

What type of ELISA is used for the ZIKV Detect 2.0 IgM Capture ELISA kit?

a) Direct ELISA

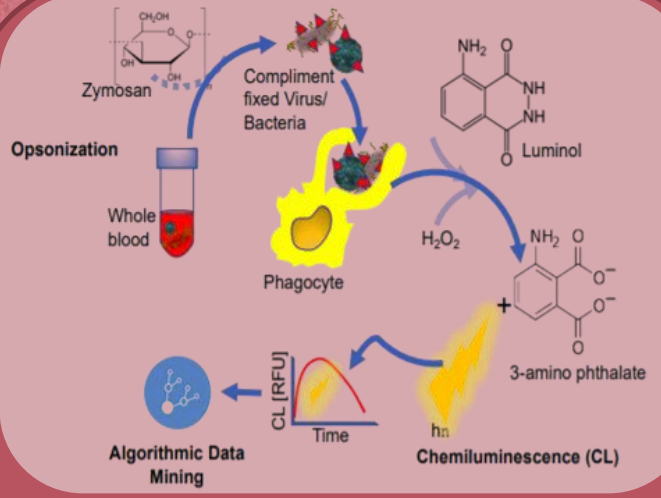
b) Indirect ELISA

c) Sandwich ELISA

d) Competitive ELISA

03 ADVIA Centaur Zika Test

- Antibody capture immunoassay using chemiluminescence detection
- Detection of IgM antibodies determined by combining in an algorithm the measurements of the ADVIA Centaur Zika Ab Assay and the ADVIA Centaur Zika IgM Assay.
- Detect positive infections 8 days after onset of symptoms or risk of exposure



04

LIAISON XL Zika Capture IgM Assay II

Purpose

fully automated serology assay for the detection of Zika virus infections

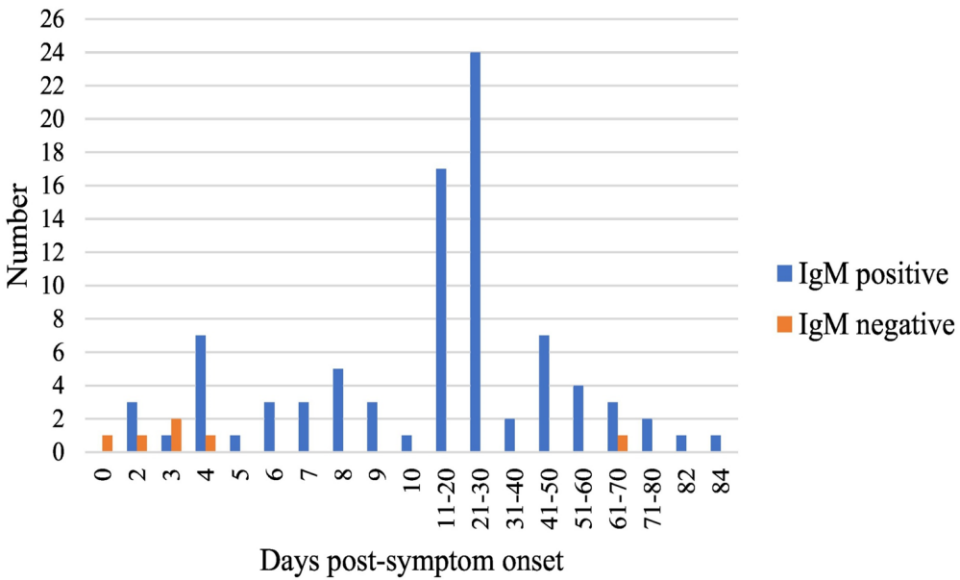
Time Frame

Specimens should be collected between 8 days and 10 weeks after onset of symptoms or risk of exposure

Results

yields results in as little as 37 minutes

Performance Evaluation



The evaluation of the LIAISON® XL Zika Capture IgM on a panel of 106 samples from patients with certain and probable ZIKV infections from 0 to 84 days pso revealed a sensitivity of 92.5%

05

DPP Zika IgM Assay System

Purpose

Detection of Zika virus IgM antibodies in fingerstick whole blood, EDTA venous whole blood, EDTA plasma and serum

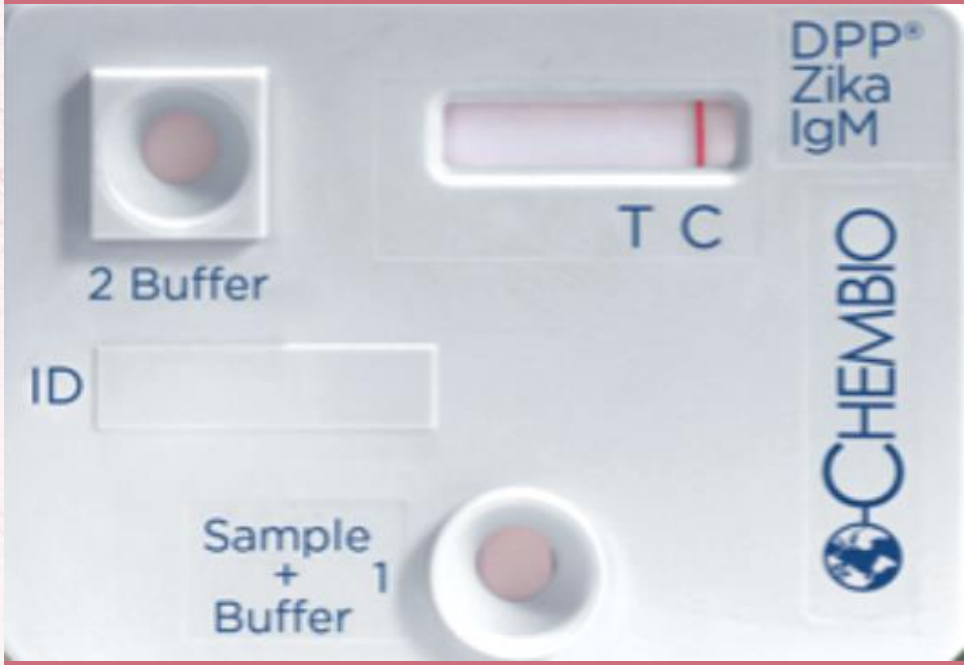
Time Frame

Detects Zika virus IgM antibodies from 4 days up to 12 weeks post-onset of symptoms

Results

15 Minute Rapid Test for Detecting Zika Virus

Device



Small sample volume: only 10 µl

Question 12

How fast can the DPP Zika IgM Assay System detect Zika virus infection?

a) 37 minutes

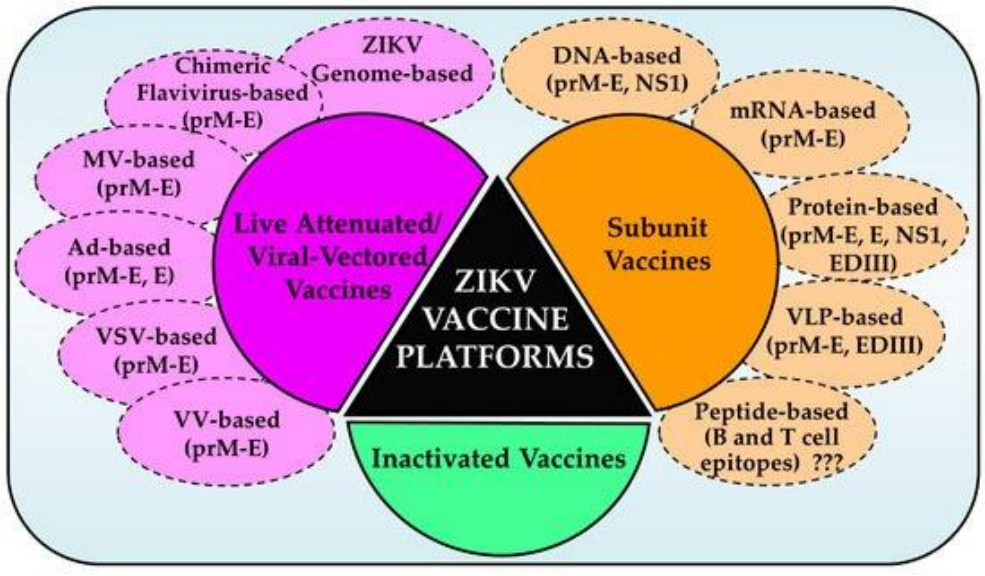
b) 4 days

c) 2 hours

d) 15 minutes

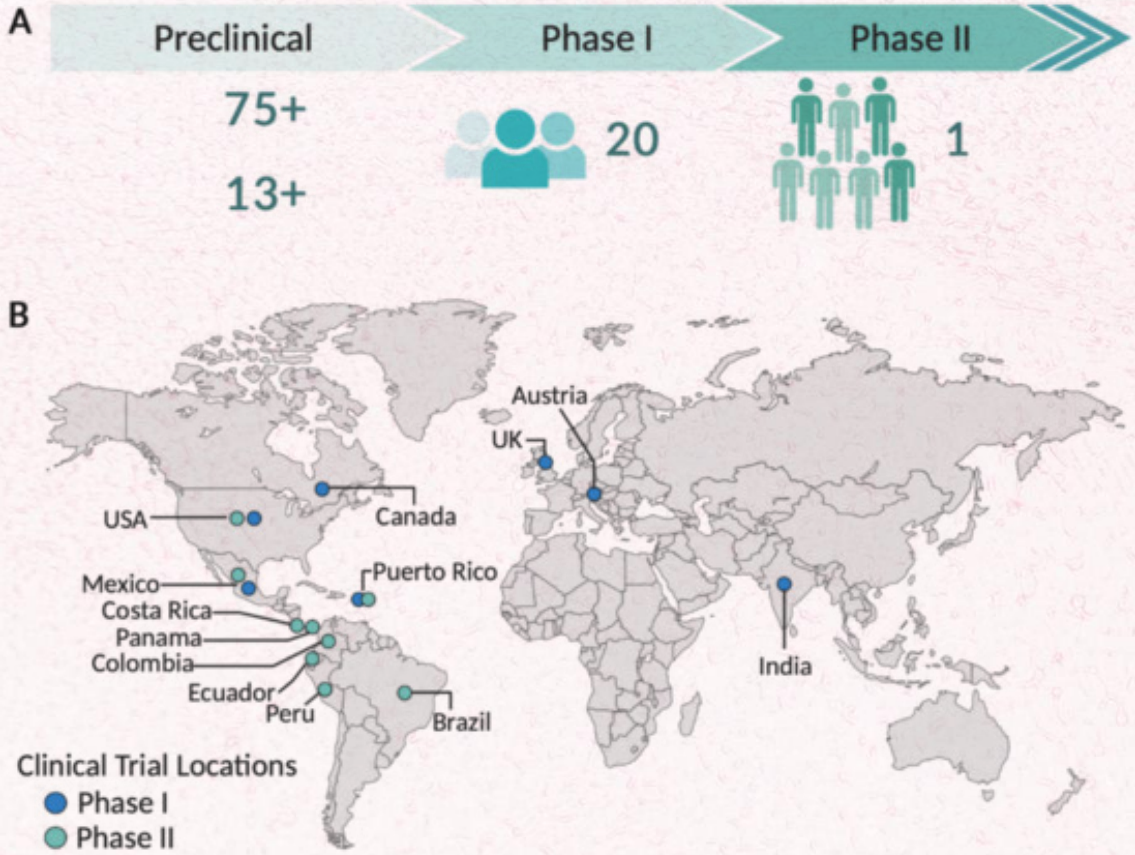
TOPIC 06
Vaccination & Drug Targets

Vaccine Development



1. Inactivated
2. Subunit
3. Live-attenuated

Clinical Trials



- Over the past 5 years, multiple vaccine platform technologies have been used in the design of vaccine prototypes
- (A) Number of Zika vaccine candidates tested in preclinical trials and in phase I and II clinical trials in humans.
- (B) phase I and II clinical trial sites

PHASE 2 CLINICAL TRIAL (04.12.20)

Vaccine: VRC 705 – DNA vaccine

SAMPLE SIZE

- 2428 participants
- 15 Years to 35 Years
- multicenter, randomized study

GOAL

To evaluate safety, immunogenicity, and efficacy of a 3-dose vaccination regimen with the Zika virus

DOSE

4 mg or 8 mg dose of vaccine split between 2 or 4 injections.

2 years and 6 Months

Experimentation time

80%

SUCCESS RATE

RESULTS

Antibody response as measured by ZIKV neutralization antibody (NAb) assay. Neutralizing activity is reported as the dilution of sera required to neutralize eighty percent of infection events (EC80)

Question 13

How many Vaccine candidates are in phase I clinical trials ?

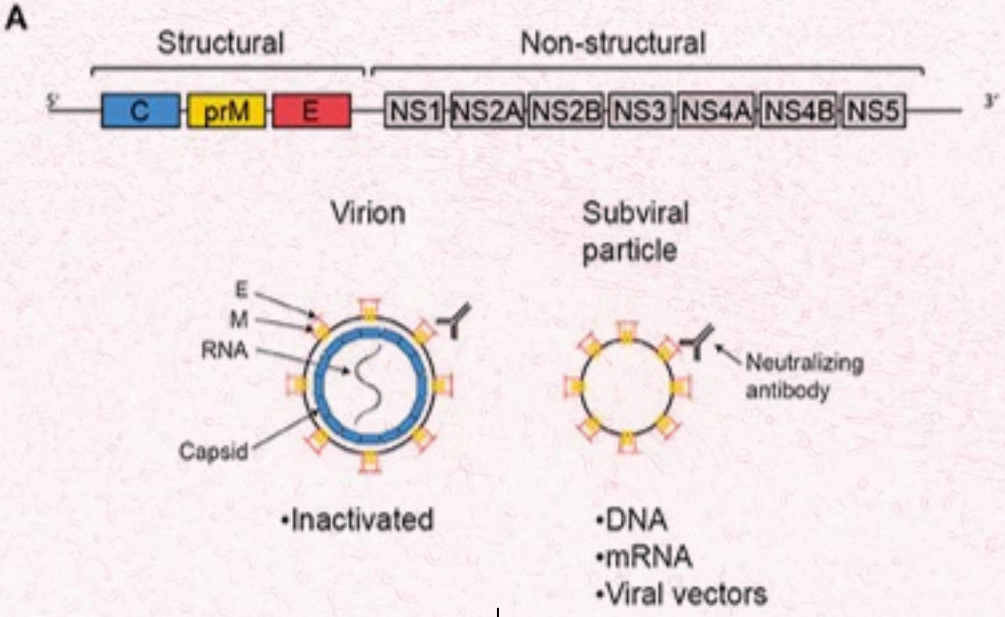
a) 75+

b) 20

c) 1

d) None

Drug Targeting



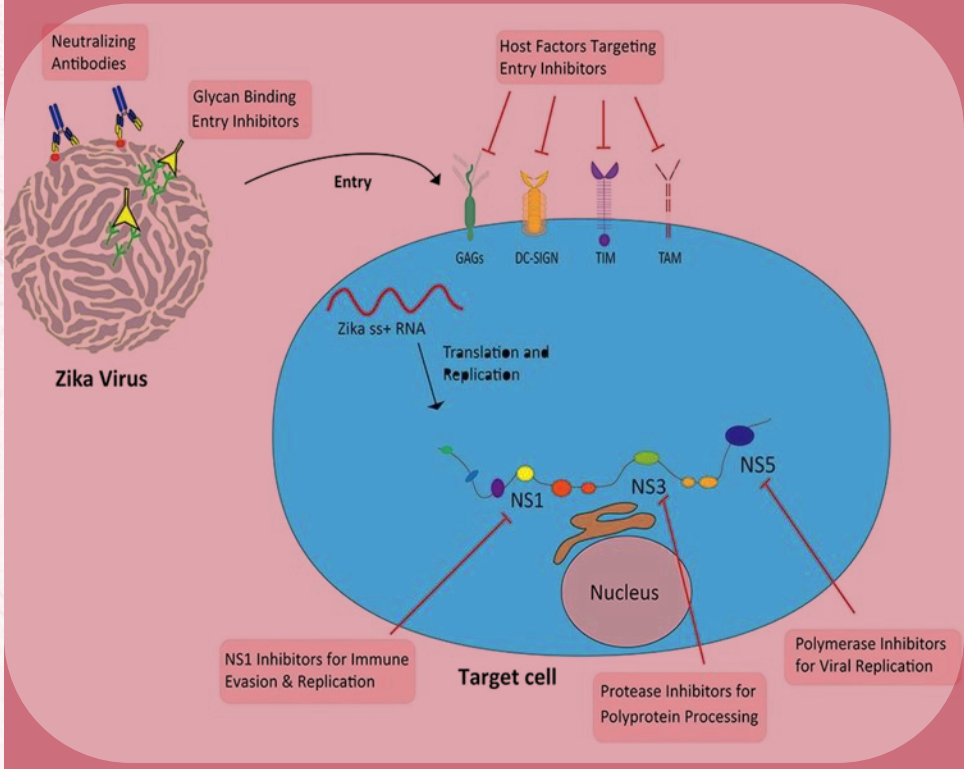
Zika genome encodes the structural genes C, prM, and E and the non-structural genes NS1-5

The E protein represents the immunodominant antigen, neutralizing antibodies against which are protective

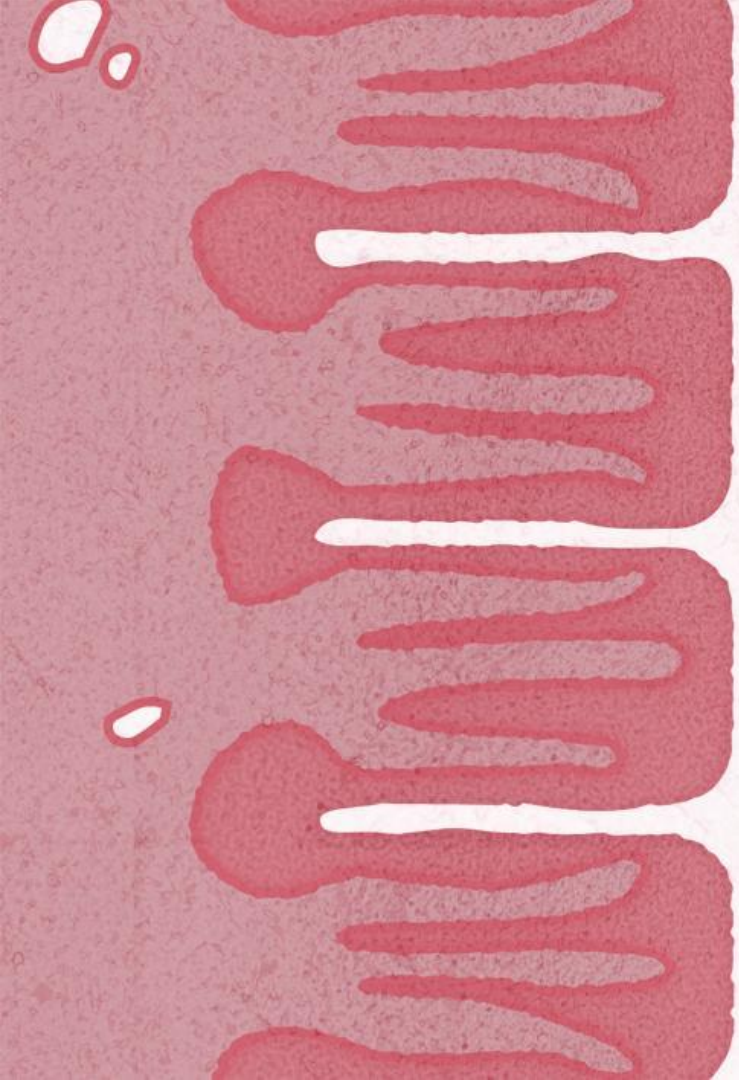
Advanced vaccine candidates differ in their platform technology, but ultimately all present immunodominant antigen either in the context of a full virion or a sub-viral particle

Potential Therapeutic Targets

- Interactions between ZIKV envelope glycoprotein E and cellular receptors mediate the viral fusion and entry to the target cell
- Blocking these interactions can inhibit viral entry into the cell
- Non-structural proteins play essential roles in viral replication cycle and potentiate for therapeutic interventions



Overview of the different stages of inhibitors that can be used to prevent ZIKV entry and replication.

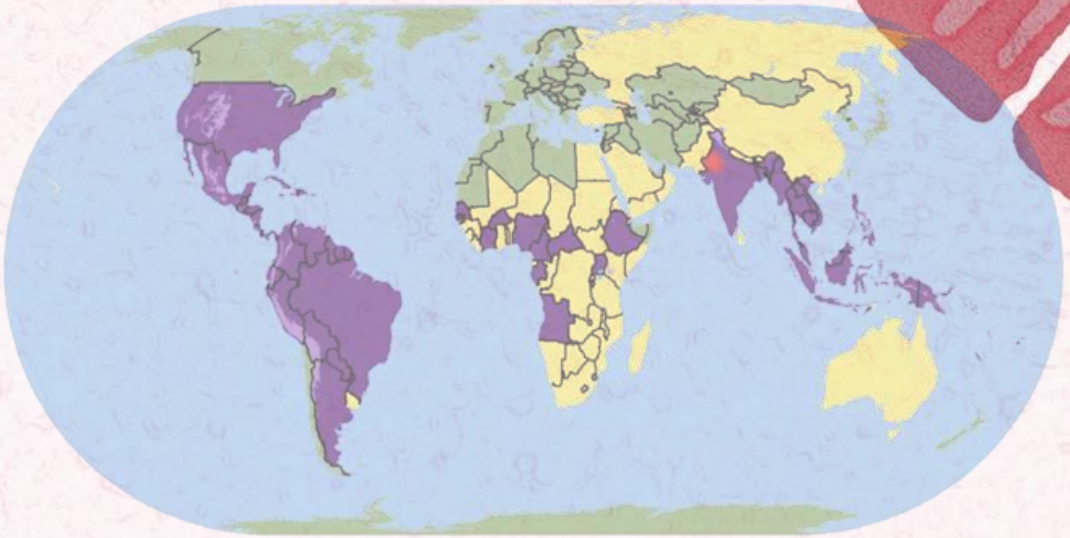


TOPIC 07

Prevalence & Conclusion

Prevalence of Zika Virus Today

- Cases
- Current situation
- Direction



Zika Virus 5 Years Later

- Five years later, despite advances in the understanding of transmission, pathogenesis, a lot is still unknown about Zika's emergence as the cause of an unexpected epidemic centred in Brazil.

- From 2017 to 2020, the annual recorded Zika cases has dropped from ~1100 to 51, respectively.

Impact of COVID-19

- COVID-19 pandemic has impacted efforts to prevent and respond to the Zika virus.
- Mosquito surveillance and control activities have been reduced in some countries, including the United States, due to COVID-19 prevention efforts.
- According to Susan Hills, a medical epidemiologist in the CDC's Division of Arboviral Diseases, at least nine Zika vaccine candidates are currently in development.
- ***Ref:*** ***Impact of COVID-19 on ZIKV***

Conclusion

- Zika virus has highlighted the threat of pandemics and their effect on the world through unprecedented rapid emergence.
- Several challenges need to be addressed.
- An estimated 3.6 billion people currently live in at risk areas.
    - Developments in technologies such as CRISPR along with vaccine development and effective monitoring of areas will see the management and possible eradication of Zika in years to come.