



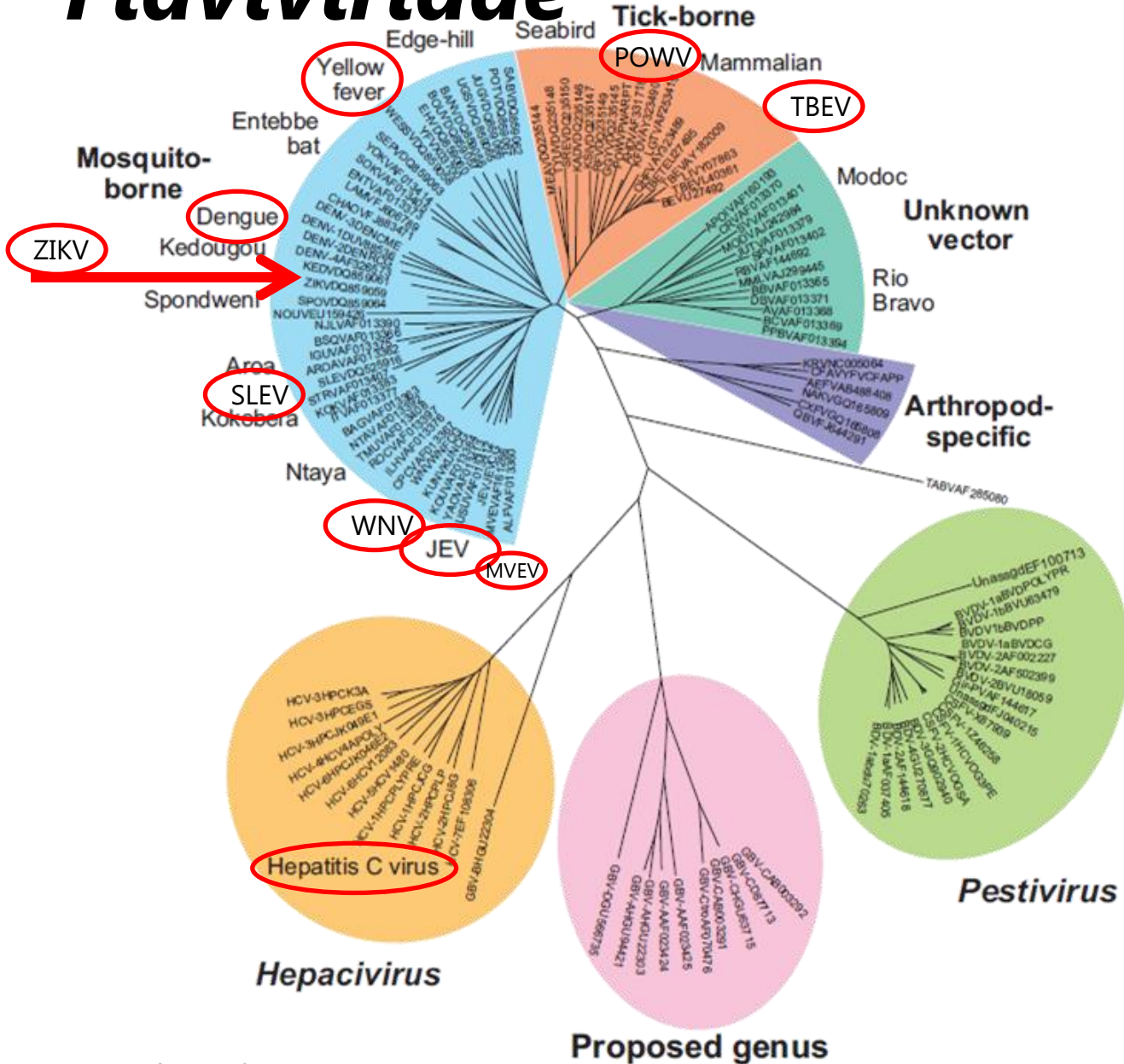
BUITENGEWOON

Flavivirus serologie

Jean-Luc Murk, arts-microbioloog

Flaviviridae

Mogelijk 'grootste'
Familie onder de virussen



0.1

Flaviviridae

Serogroup	Virus	Abr.	Vector	Host	Geographical distribution
Zika virus					
Dengue virus	Dengue virus	DENV	Mosquito	Primates, humans	Asia, Africa, Americas
Japanese encephalitis	Japanese encephalitis virus	JEV	Mosquito	Ardeid birds, pigs	South and South-East Asia, Oceania
	West Nile virus	WNV	Mosquito	Birds	North and South America, South and Eastern Europe, South-East Asia, Oceania
	Kunjin virus				
	St. Louis encephalitis virus	SLEV	Mosquito	Birds	Americas
	Murray Valley virus	MVEV	Mosquito	Ardeid birds	Oceania
Mammalian tick-borne virus group I	Kyasanur Forest disease virus	KFDV	Tick	Small mammals, humans	South-East and Western Asia
	Alkhurma hemorrhagic fever virus	AHFV	Tick	Small mammals	Western Asia
	Tick-borne encephalitis virus	TBEV	Tick	Small mammals, birds	Central, Northern and Eastern Europe, and Asia
Powassan virus					
Ntaya virus	Ilheus virus	ILHV	Mosquito	Birds	Central and South America
Yellow fever	Yellow fever virus	YFV	Mosquito	Primates, humans	Sub-Saharan Africa and South America

NB: hepatitis C virus (hepacivirus) wordt niet besproken in deze presentatie

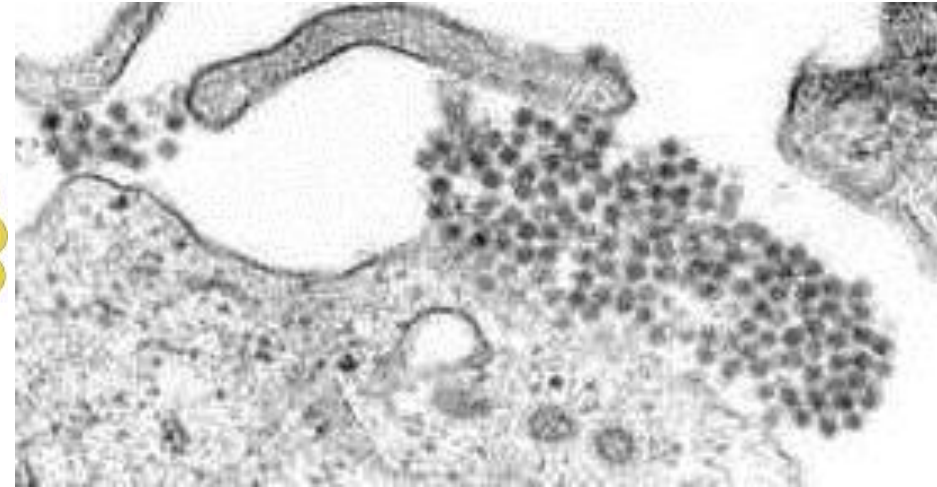
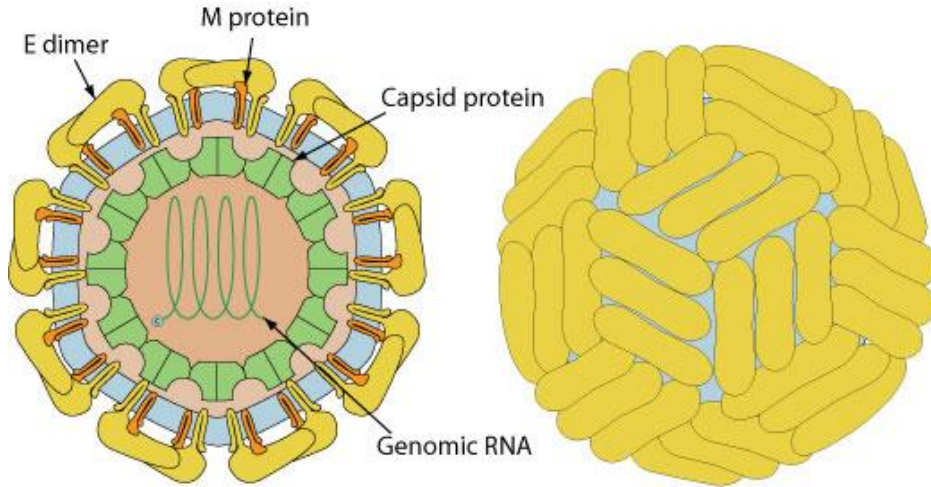
Flaviviridae

Virus	Symptoms ^a	Incubation period in days	Vaccine
Dengue virus	FD, HS, AR	4-7 (3-14)	Yes
Japanese encephalitis virus	FD, NS	5-14	Yes
West Nile virus	FD, NS, (AR)	3-5 (2-14)	No
St. Louis encephalitis virus	FD, NS	2-21	No
Murray Valley virus	FD, NS	1-28	No
Kyasanur Forest disease virus	FD, HS, conjunctivitis, pneumonia	3-8	Yes
Alkhurma hemorrhagic fever virus	FD, HS	3-12	No
Tick-borne encephalitis virus	FD, NS, (HS)	7-14	Yes
Ilheus virus	FD, NS	Unknown	No
Yellow fever virus	FD, HS, hepatitis	3-6	Yes
Zika virus	FD, NS, AR, Cong	2-14	No

Ziektebeelden:

- Frequent asymptomatisch!
- Aspecifieke koorts (FD)
- 'Hemorragische' koorts (HS) / shock syndroom
- Neurologische ziekte (NS)
- Artralgie en of Rash (AR)
- Overige presentaties: hepatitis, enz
- Congenitale infecties (ZIKV... HCV)

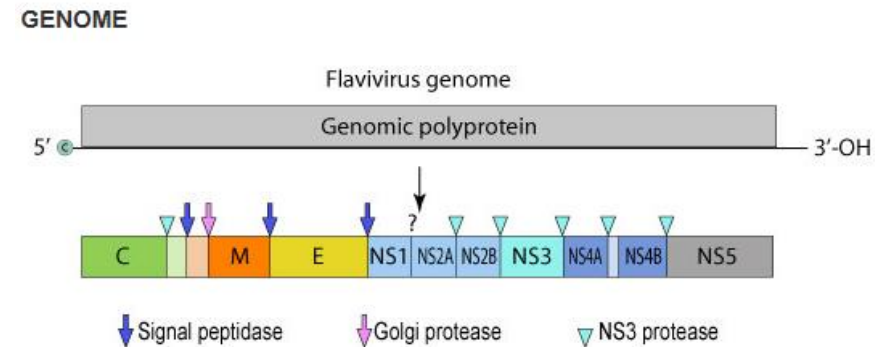
Flaviviridae



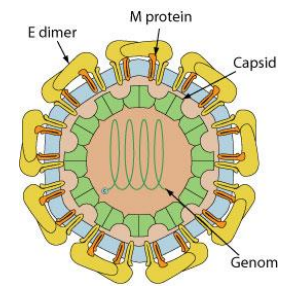
ssRNA(+) virus
envelop

Diameter: 50 nm

Genoom: ~9,4 - 13 kb

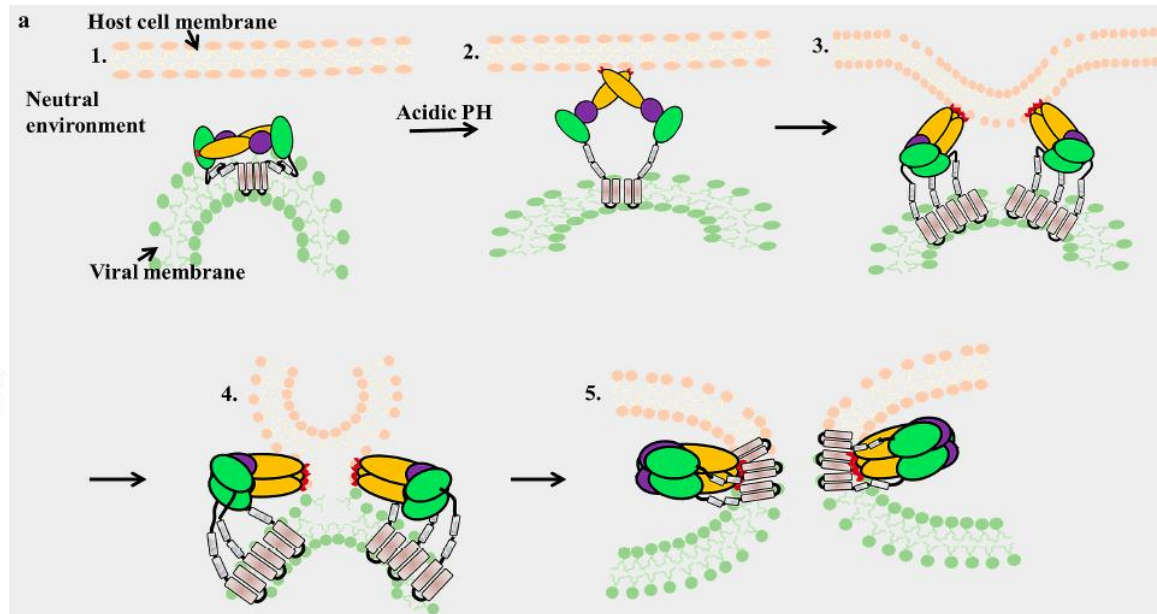
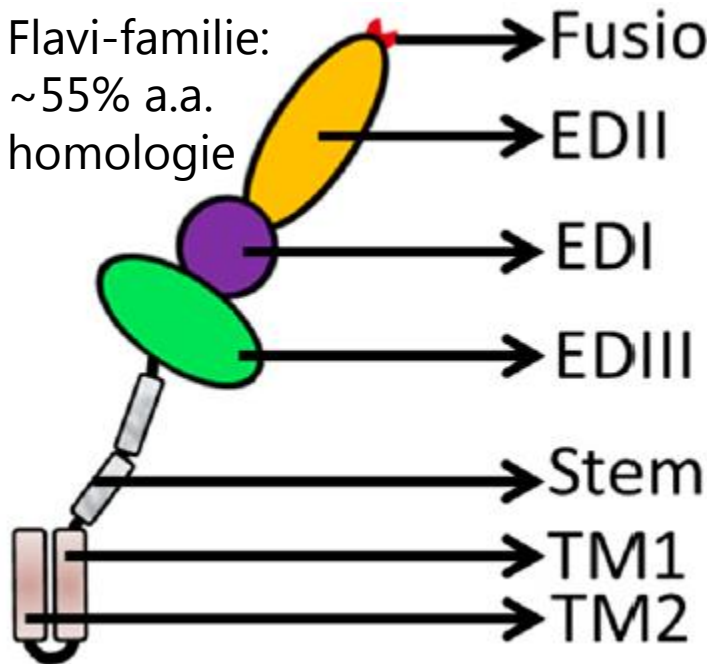


Flaviviridae: E-eiwit



Essentieel voor het virus: receptor binding, membraan fusie, uncoating (infectie vd cel), haemagglutinatie. Bevat meerdere zeer geconserveerde stukken in ieder domein!

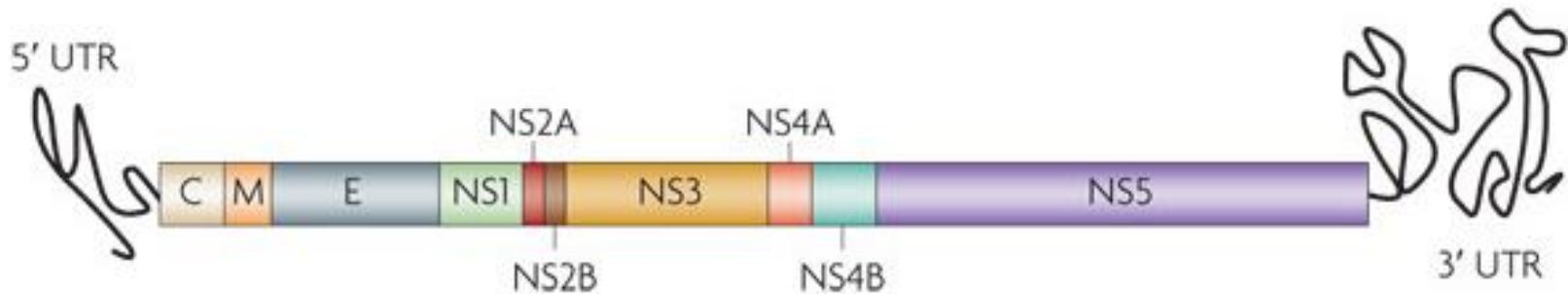
Flavi-familie:
~55% a.a.
homologie



ED1: target van type specifieke, niet-neutraliserende antistoffen
ED2: target van kruisreagerende antistoffen & neutraliserende antistoffen
ED3: target van veel antistoffen, target van type specifieke neutraliserende antistoffen, maar ook kruisreagerende (neutraliserende) antistoffen

Flaviviridae: NS1-eiwit

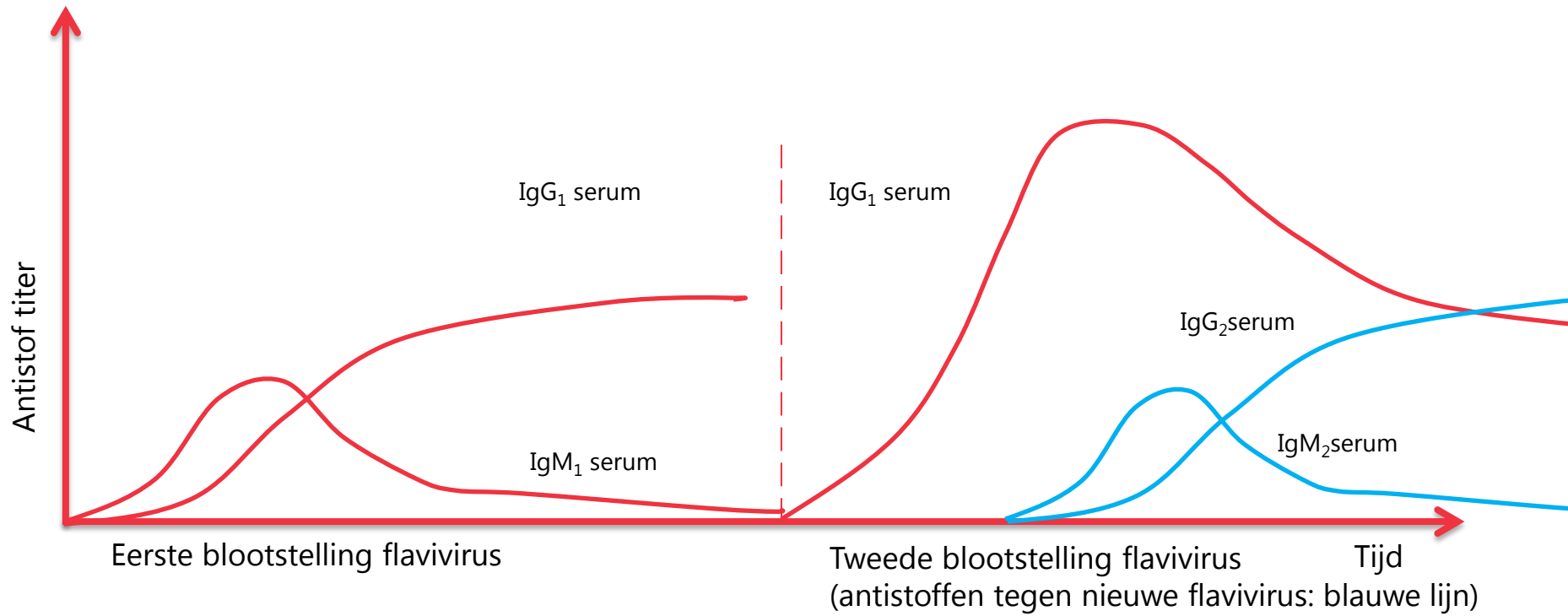
- ~352 aminozuren
- verschillende vormen: membraan gebonden, intracellulair, vrij in bloed
- Hoog immunogeen
- Zou meer specifieke epitopen dan het E eiwit bevatten, maar ook grote homologie tussen flavivirus sp
- 'antigeen in bloed correleert met virale replicatie'



Humane antistof respons:

- cocktail aan antistoffen tegen verschillende virusantigenen / epitopen
 - virus-type specifieke antistoffen
 - antistoffen tegen geconserveerde gebieden: kruisreactiviteit
 - neutraliserende en niet-neutraliserende antistoffen (waaronder kruisreagerende neutraliserende antistoffen)
 - 'antigenic sin'
- In de regel levenslange bescherming voor infectie met zelfde virus (NB: uitzonderingen, bijv HCV)

Antigenic sin



Kruisreactiviteit: een illustratie

Lanciotti 2008

Table 1. IgG and IgM testing with heterologous flaviviruses of patients infected with ZIKV, Yap State, Micronesia, 2007*

Patient	Days after onset	IgG			IgM			
		ZIKV	ZIKV	DENV	YFV	JEV	MVEV	WNV
Primary flavivirus ZIKV								
822a	5	1.5	23.2	1.3	1.4	1.7	1.1	–
822b	10	1.2	39.5	1.2	1.0	2.4	1.2	–
822c	24	3.3	13.1	2.7	0.63	1.8	1.3	–
830a	2	1.1	1.3	4.4	0.48	4.4	2.9	–
830b	21	1.8	16.3	1.9	0.63	1.3	1.6	–
849a	3	1.5	4.5	0.92	0.95	1.2	0.66	–
849b	18	3.0	18.2	2.2	1.0	2.7	1.5	–
862a	6	1.9	25.4	1.7	1.1	1.8	1.0	–
862b	20	2.6	15.4	2	1.1	2.3	1.1	Eq
Secondary flavivirus ZIKV (probable)								
817a	1	5.9	1.4	1.7	0.8	1.7	0.7	–
817b	19	5.7	8.1	5.1	2.1	1.7	1.0	–
833a	1	3.4	1.7	3.7	1.0	2.8	1.3	–
833b	19	8.2	3.1	2.3	0.9	2.5	1.3	–
844a	2	3.8	3.8	6.8	2.0	21.5	0.7	–
844b	16	8.5	12.7	14.9	7.0	42.9	1.6	–
955a	1	5.0	1.8	3.7	1.0	3.4	2.4	Eq
955b	14	26.6	10.9	3.4	0.8	1.7	4.0	Eq
968a	1	4.0	1.7	1.3	0.6	1.2	1.2	–
968b	3	12.3	20.4	2.9	0.8	0.9	2.0	–
839a	3	1	0.92	3.4	0.7	2.7	2.1	–
839b	20	4.9	17.2	2.2	2.1	1.9	1.8	–
847a	5	0.9	0.94	4.1	4.1	2.3	1.3	–
847b	8	14.1	21.5	1.4	3.3	1.1	2.6	–

*Ig, immunoglobulin; ZIKV, Zika virus; DENV, dengue virus type 1–4 mixture; YFV, yellow fever virus; JEV, Japanese encephalitis virus; MVEV, Murray Valley encephalitis virus; WNV, West Nile virus; –, negative. Eq, result in equivocal range of the assay. IgG and IgM testing was conducted by ELISA except for WNV, which was tested by microsphere assay; ELISA values are patient optical densities divided by negative control optical densities; <2, negative; 2–3 equivocal; >3 positive.

Kruisreactiviteit: een illustratie

Lanciotti 2008

Table 2. Neutralization testing with heterologous flaviviruses of patients infected with ZIKV, Yap State, Micronesia, 2007*

Patient	Days after onset	PRNT ₉₀ titer									
		ZIKV	DENV1	DENV2	DENV3	DENV4	JEV	YFV	WNV	SLEV	MVEV
Primary flavivirus ZIKV											
822a	5	320	<10	<10	<10	<10	<10	<10	<10	<10	<10
822b	10	2,560	10	10	10	10	<10	<10	<10	<10	<10
822c	24	5,120	10	10	10	10	<10	<10	<10	<10	<10
830a	2	<10	<10	NT‡	NT	NT	NT	NT	NT	NT	NT
830b	21	2,560	<10	<10	<10	<10	<10	<10	<10	<10	<10
849a	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
849b	18	10,240	<10	<10	<10	<10	<10	20	<10	<10	<10
862a	6	320	<10	<10	<10	<10	<10	<10	<10	<10	<10
862b	20	2,560	10	10	<10	<10	<10	<10	<10	10	<10
Secondary flavivirus ZIKV (probable)											
817a	1	80	80	160	320	160	<10	<10	<10	40	40
817b	19	10,240	2,560	20,480	5,120	5,120	20	320	160	1,280	640
833a	1	160	320	80	40	20	<10	<10	<10	<10	<10
833b	19	81,920	20,480	5,120	5,120	1,280	<10	<10	80	320	320
844a	2	20	1,280	640	320	160	<10	<10	5	20	20
844b	16	10,240	40,980	10,240	5,120	1,280	5	<10	160	640	640
955a	1	40	1,280	640	160	320	<10	<10	<10	20	20
955b	14	163,840	81,920	20,480	10,240	5,120	10	<10	640	2,560	1,280
968a	1	80	320	320	80	40	<10	<10	<10	40	20
968b	3	10,240	640	640	160	160	<10	<10	10	40	20
839a	3	<10	<10	10	<10	<10	<10	40	<10	<10	<10
839b	20	10,240	40	320	80	80	<10	640	40	80	80
847a	5	<10	<10	<10	<10	<10	<10	640	<10	<10	<10
847b	8	2,560	40	320	160	40	<10	1,280	80	320	320

*PRNT₉₀ titer, 90% plaque reduction neutralization test titer; ZIKV, Zika virus; DENV, dengue virus; JEV, Japanese encephalitis virus; YFV, yellow fever virus; WNV, West Nile virus; SLEV, St. Louis encephalitis virus; MVEV, Murray Valley encephalitis virus; NT, not tested (sample depleted).

- Diagnostische tools:
 - PCR (virus isolatie)
 - IgM & IgG (IgA)
 - NS1 antigeentest
 - Virus neutralisatie test
 - Aviditeitstesten

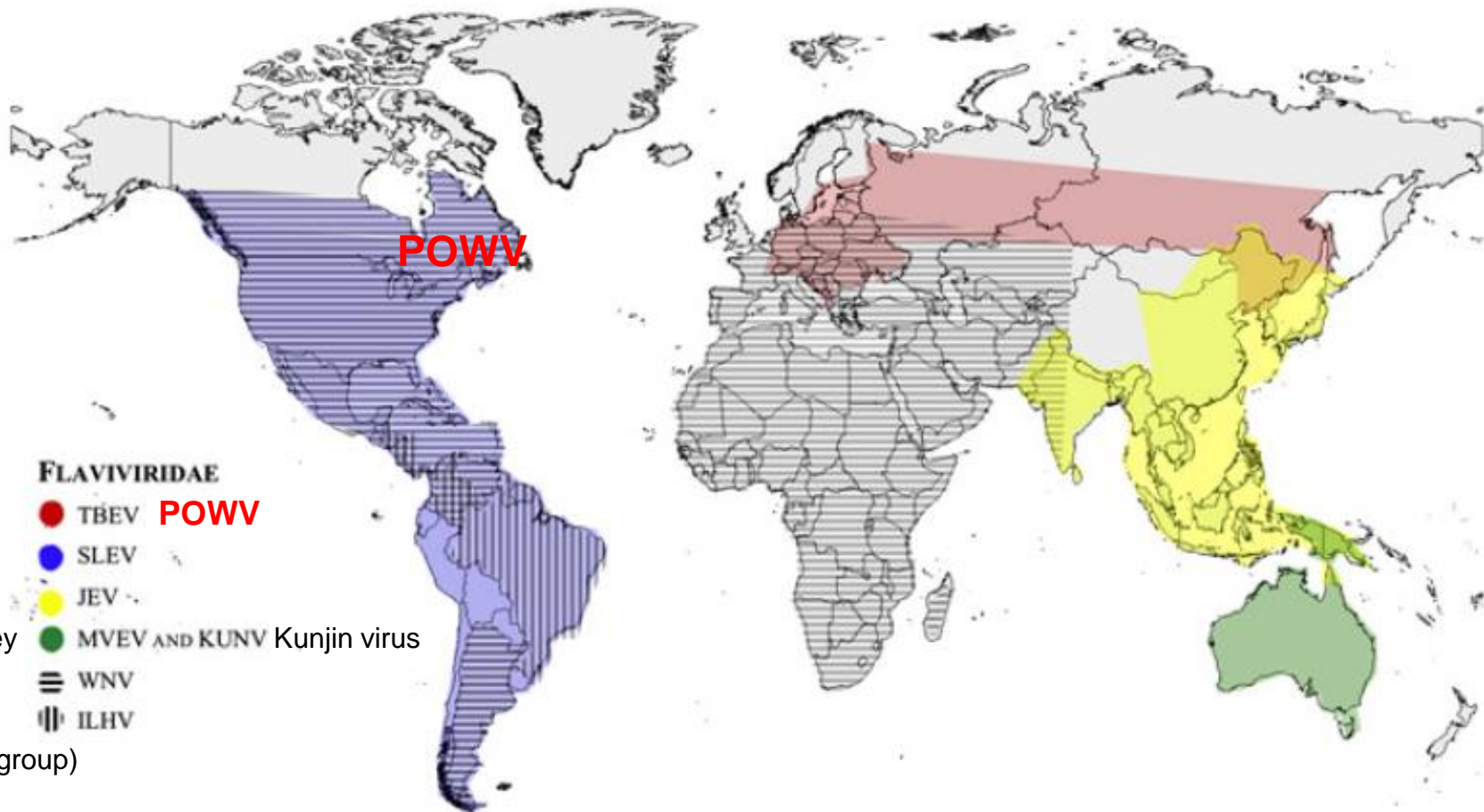
Flavivirus diagnostische tools

- Diagnostische tools:
 - PCR (virus isolatie)
 - IgM & IgG (IgA)
 - NS1 antigeentest
 - Virus neutralisatie test

- ‘In opkomst’:
 - Aviditeitstesten
 - Assays met type specifieke epitopen
 - Multiplex testen

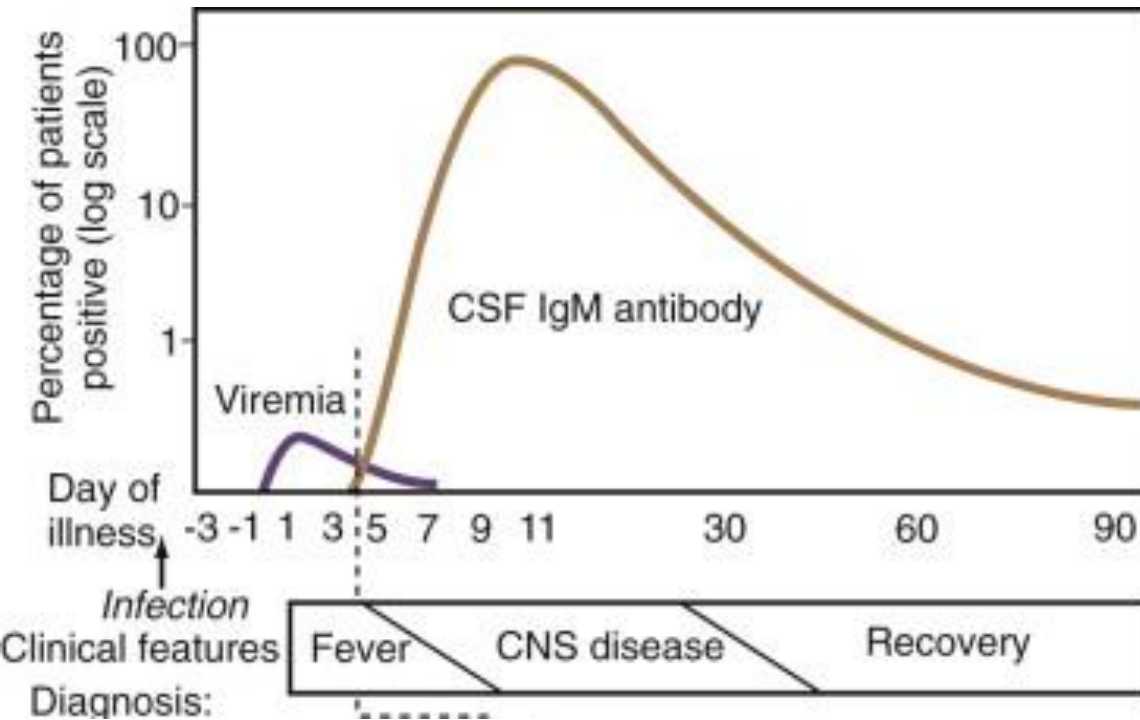
Diagnostiek CZS infecties

Neuroinvasieve flavivirussen



NB: dengue soms ook neuro-invasief

WNV diagnostiek



IgM:

Liquor diagnostiek!

IgM vaak eerder in li dan serum

Vroeg id ziekte nog negatief

half jaar of langer aantoonbaar

IgG:

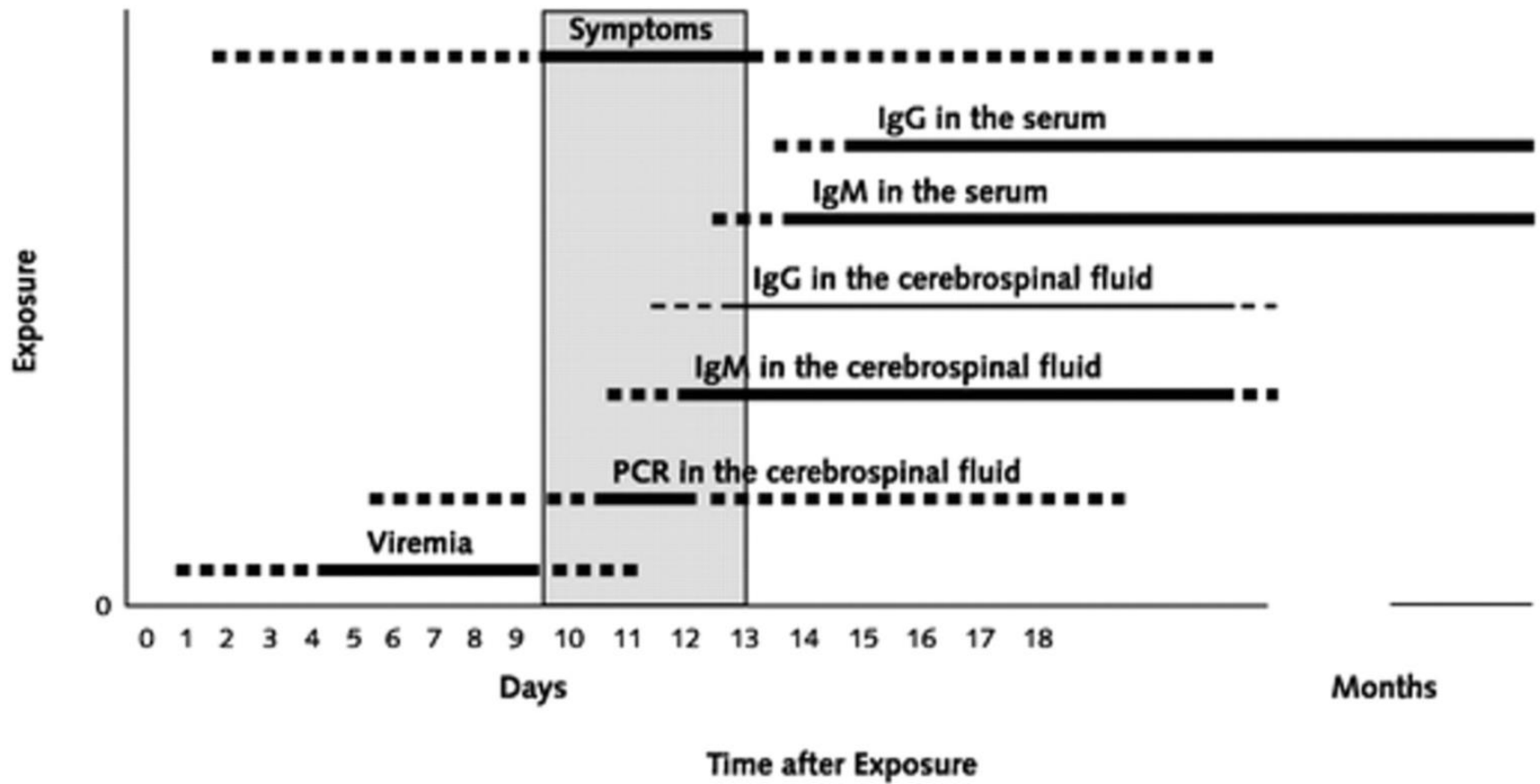
Marker doorgemaakte infectie

kruisreactiviteit met oa dengue

PCR

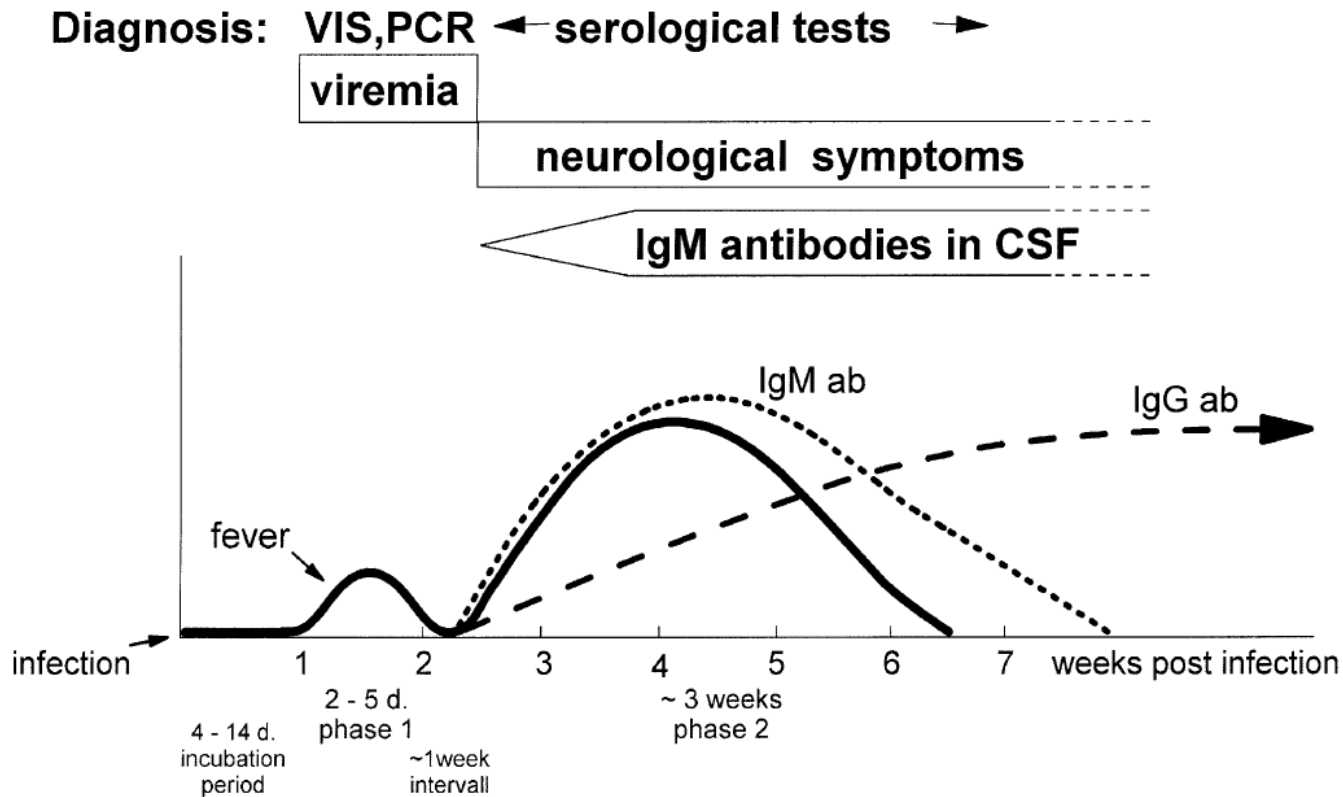
Antibody detection
IgM ELISA of CSF
~50% by hospital admission
~95% positive by day 10 of illness

WNV diagnostiek



TBEV diagnostiek

IgM eerder in serum dan liquor!
Vaak diagnose obv serum-serologie
Antistofindex / Reiber index kan zinvol zijn



TBEV diagnostiek

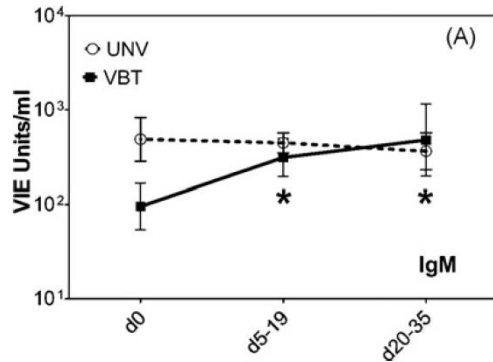
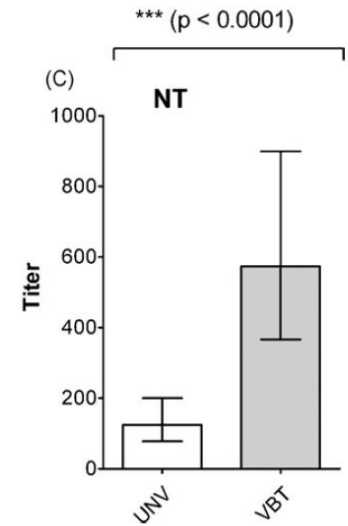
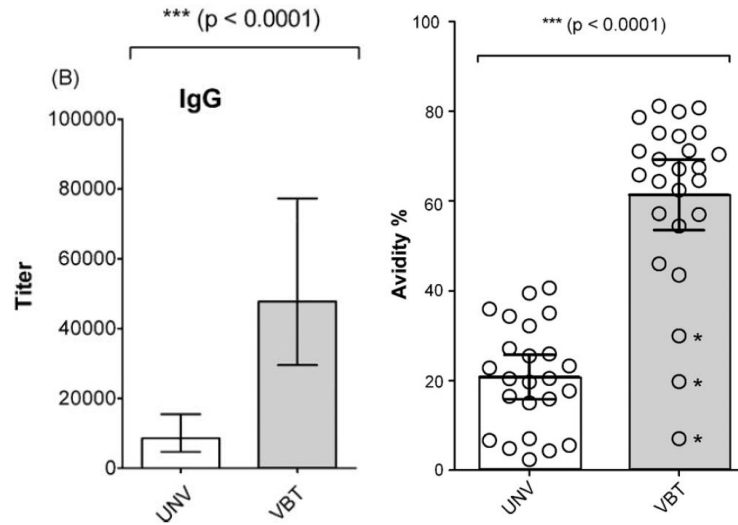
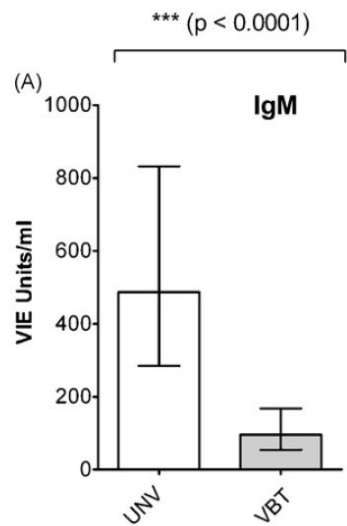
Table 3

Follow-up studies in patients with TBE: pathological findings in the CSF.

	3–7 days (n = 12)	8–14 days (n = 8)	> 14 days ^a (n = 13)
Pleocytosis in CSF	12	8	9
Blood-CSF barrier impairment	11	7	10
Oligoclonal bands	4	7	7
IgG synthesis	3	2	8
IgM synthesis	7	4	7
IgA synthesis	1	1	6
TBE-specific IgM in serum	12 100%	8 100%	8 62%
TBE-specific IgG in serum	7 58%	6 75%	13 100%
TBE-IgM-AI	9 75%	8 100%	10 77%
TBE-IgG-AI	9	8	13 100%

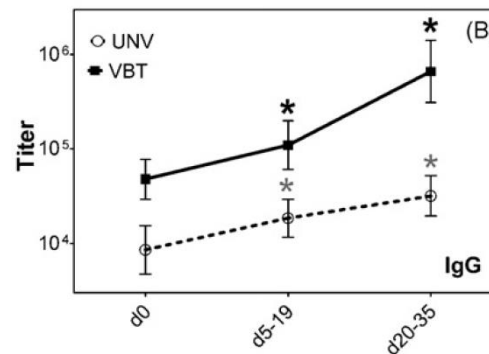
^a Median: 24 days, range: 15–120 days; AI: increased antibody index (> 2)

TBEV: serologie verloopt anders na TBEV vaccinatie!



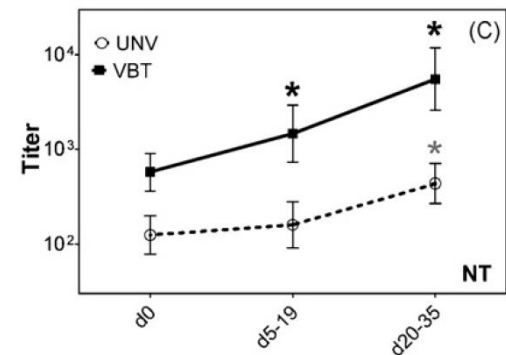
N=17 UNV
N=19 BVT

N=11 UNV
N=11 BVT



N=17 UNV
N=19 BVT

N=11 UNV
N=11 BVT

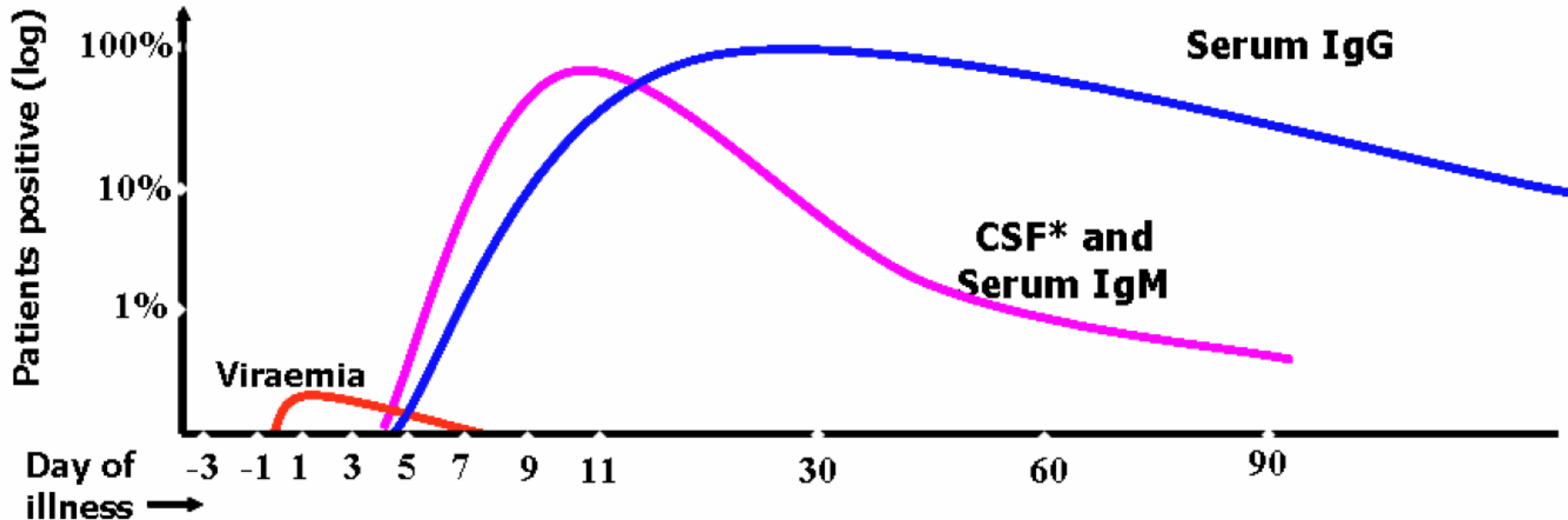


N=17 UNV
N=19 BVT

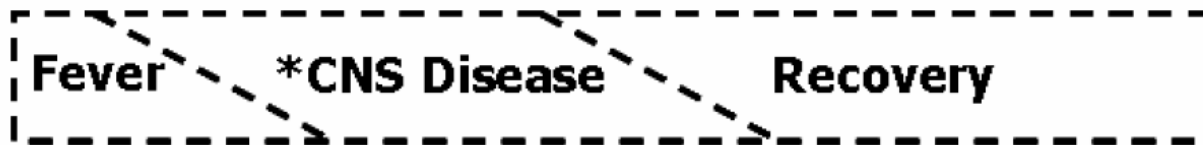
N=11 UNV
N=11 BVT

JEV diagnostiek

Vaccin falen komt voor
Onvolledige vaccinatie



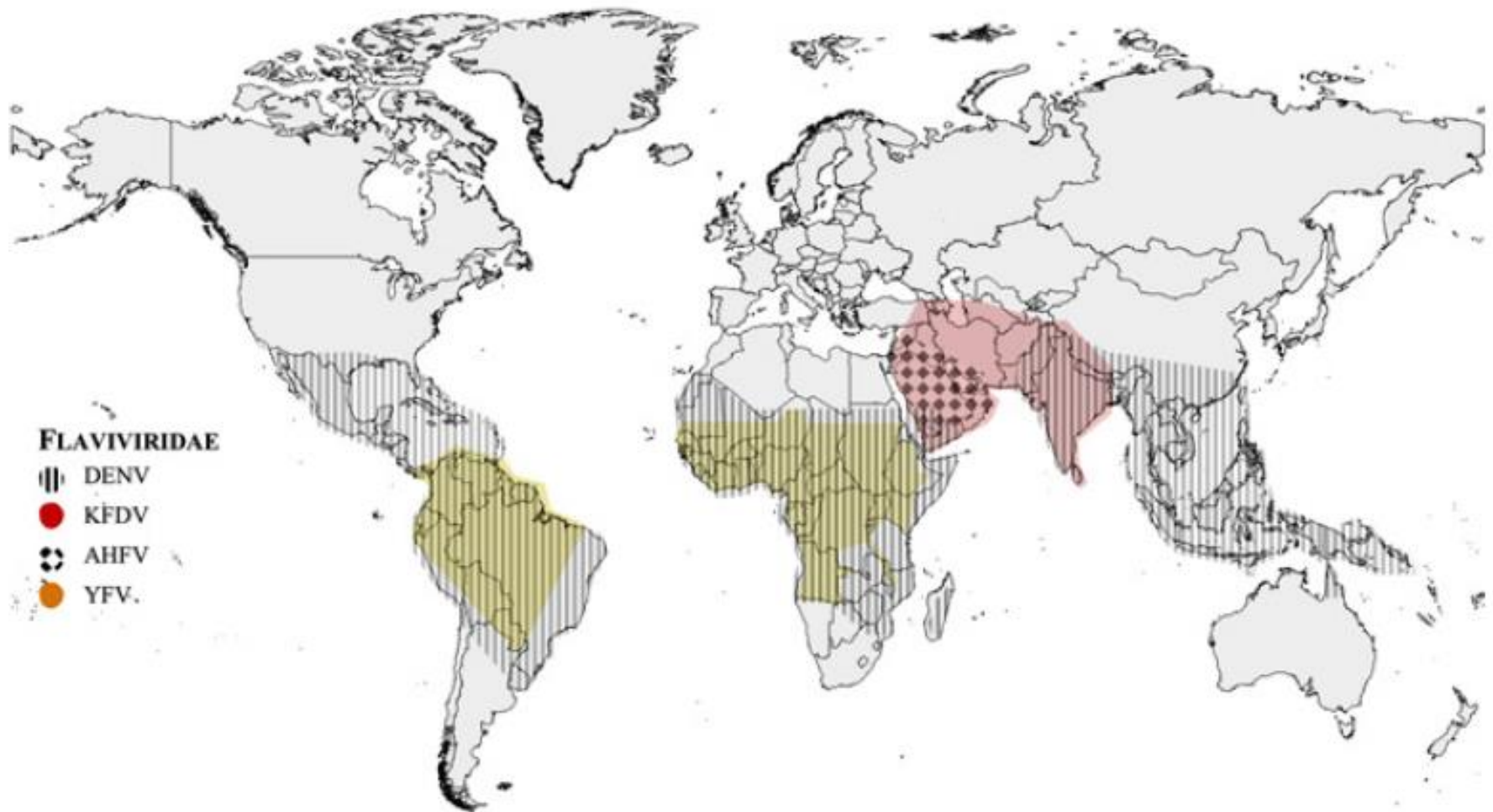
Incubation
4-14 days



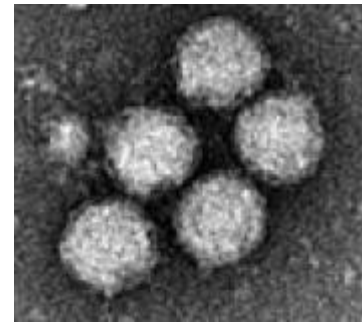
Opkomst IgM in serum en liquor min of meer gelijktijdig
Tot 4 dagen na ontstaan ziekte: IgM sensitiviteit max 75%
Na 10 dagen vrijwel 100% IgM positief
Samen testen met antistoffen tegen dengue virus!

Diagnostiek flavivirussen die hemorragische koorts veroorzaken

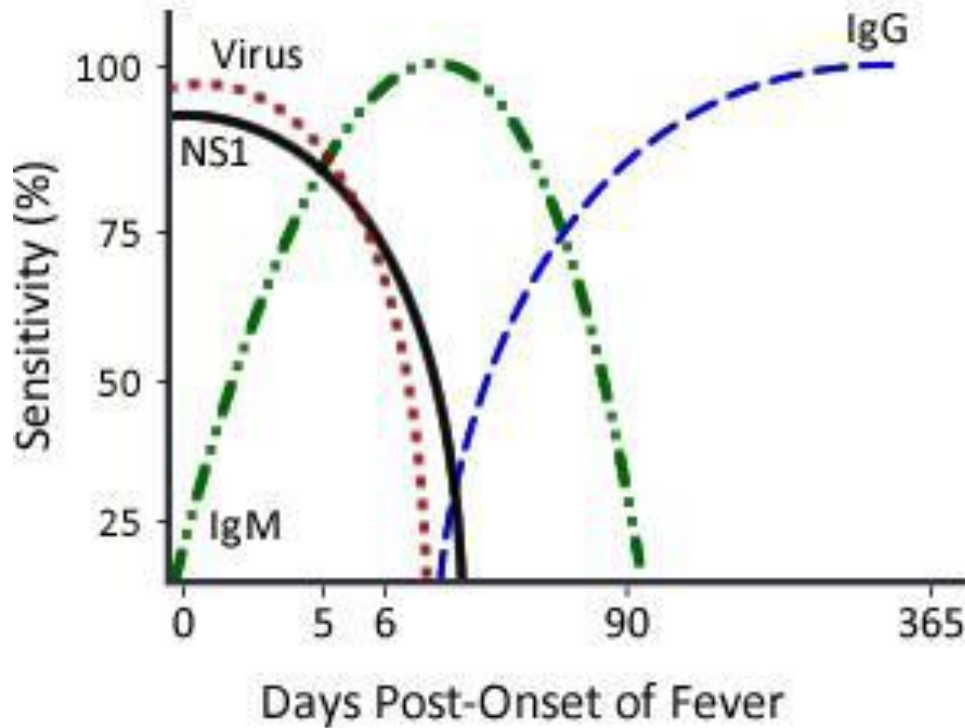
Flavivirussen met hemorragische koorts



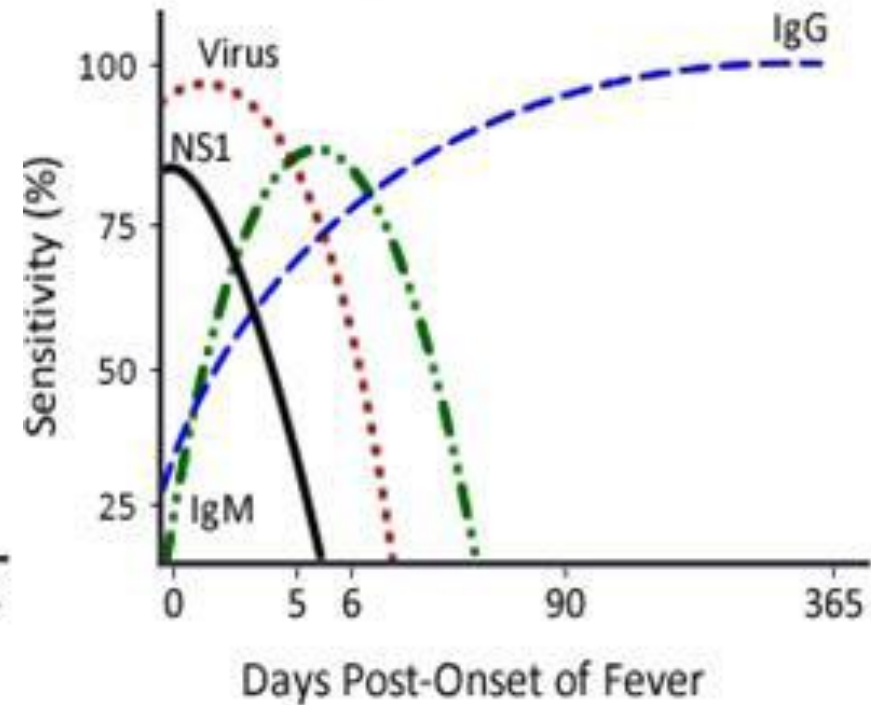
Dengue



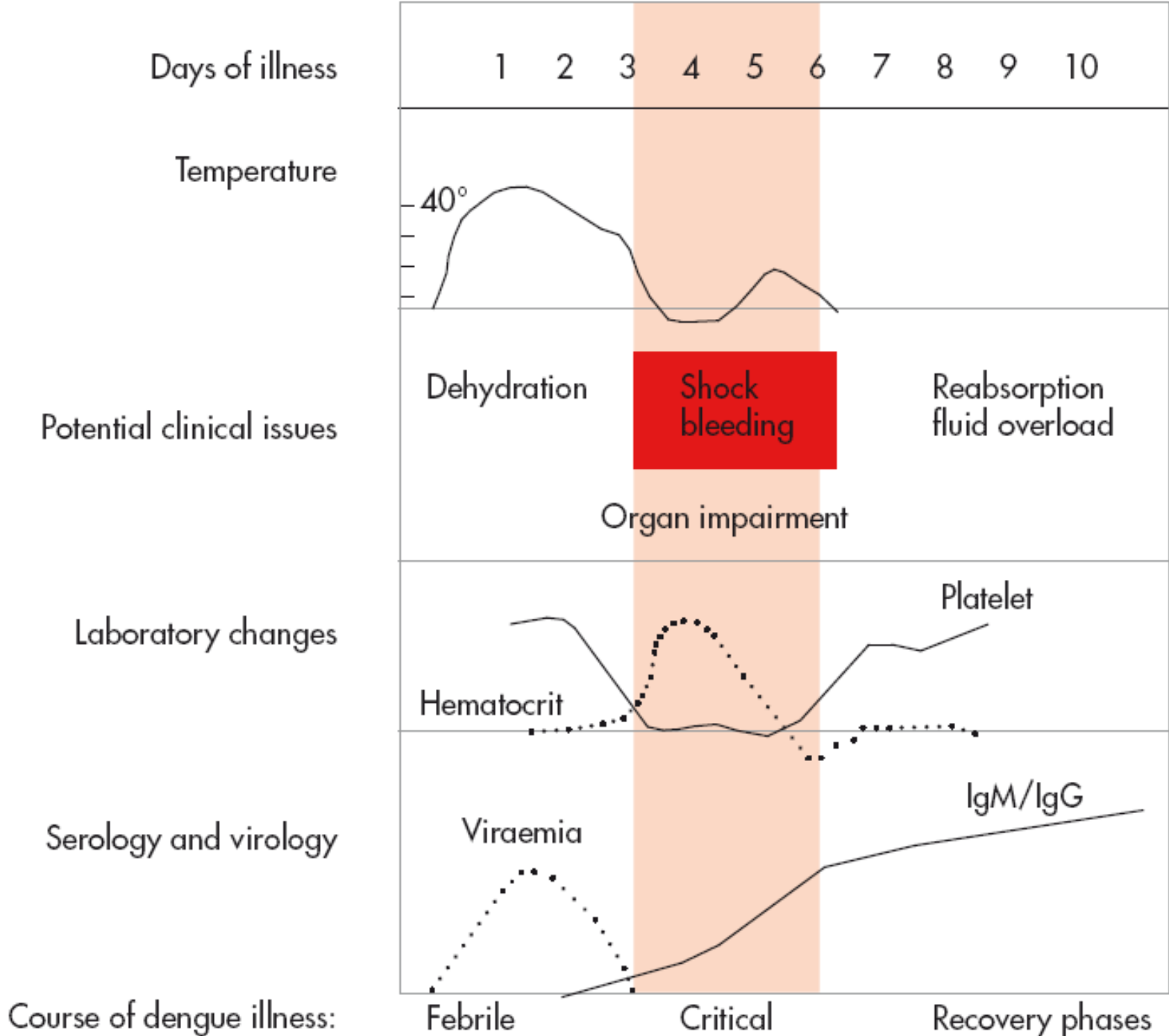
Primaire infectie



Secundaire infectie

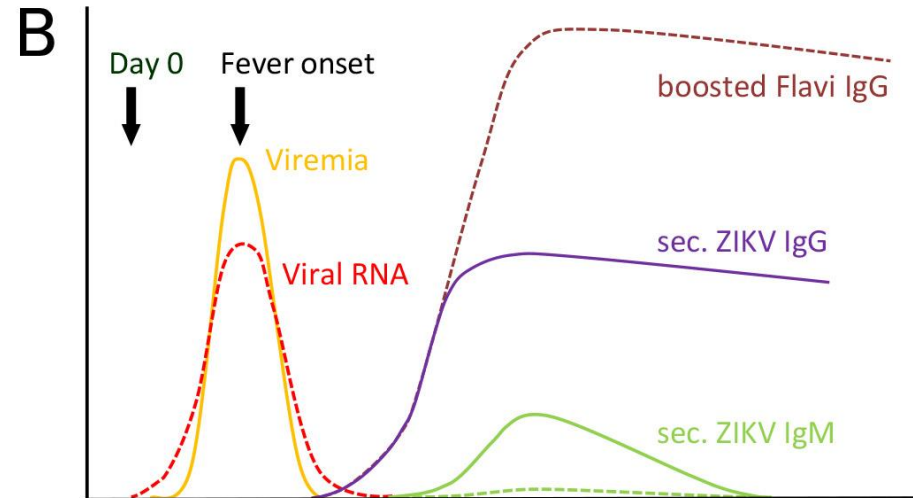
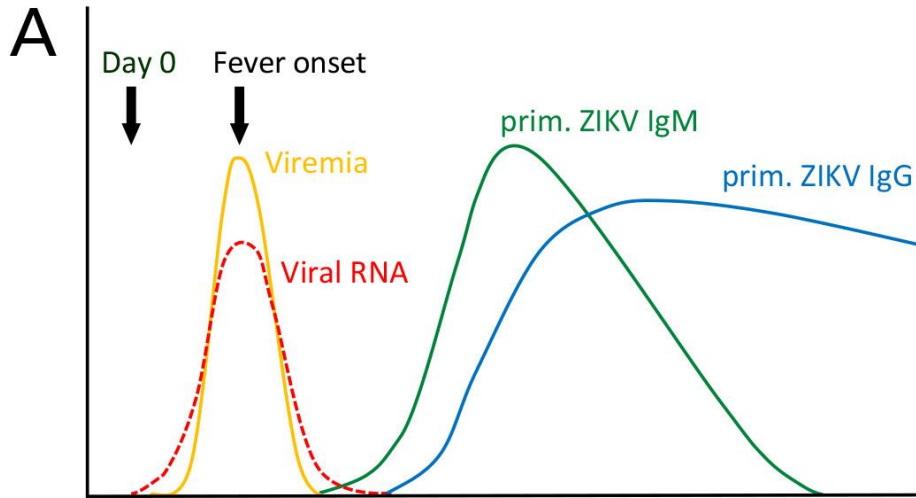


E-eiwit: 77% aminozuur homologie met TBEV & JEV



Diagnostiek zika virus

Zika virus



Tenminste altijd samen testen voor dengue (& event chikungunya)
IgM hoeft niet op te komen, bij eerder doorgemaakte flavivirus infectie!

Take home

- Serologie belangrijke tool
- Soms ingewikkelde en inconclusieve serologie
- Reisanamnese, vaccinatie historie, klinisch beeld!!
- Veel nieuwe ontwikkelingen...