

Diagnosing arbovirus infections (and Bill's holiday snaps)

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Arboviral illnesses relevant to Australia

	Alphaviruses	Flaviviruses
Polyarthralgia	Ross River virus, Barmah Forest virus, Chikungunya virus, Sindbis viru,	Kunjin virus, Kokobera virus
Fever and rash	Ross River virus, Barmah Forest,	Dengue, Kunjin
Encephalitis		Murray Valley encephalitis, Kunjin, West Nile, Japanese encephalitis
Febrile illness		Dengue, Murray Valley encephalitis KUN, WN, JE

Why diagnose arbovirus infections?

- Patient management
 - Outcomes
 - Treatment options
 - Avoid unnecessary investigations
- Public health
 - Interventions to control outbreak
 - Risks to residents or travelers
 - Understanding epidemiology and transmission
 - Future strategies

Diagnosis of arbovirus infections

- Detection of virus
 - Culture: cells, animal inoculation, mosquito inoculation
 - Antigen detection
 - Nucleic acid detection: PCR
- Antibody detection
 - IgM detection
 - IgG seroconversion
 - Rise in IgG levels

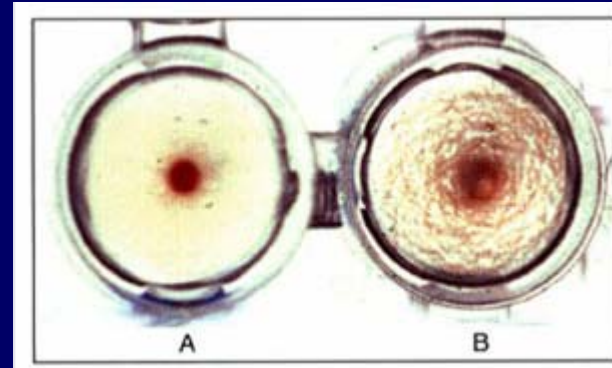
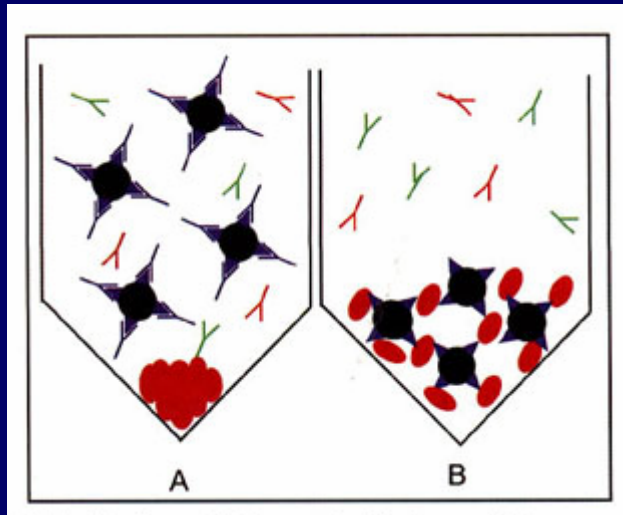
Arbovirus diagnosis: Life wasn't meant to be easy

- Clinical illnesses not distinctive
- Direct detection
 - Lack of useful culture-based methods
 - Limited PCR availability
 - Transient viraemia
- Serology
 - Cross-reactive antibody, inc. immune recall phenomena
 - Persistence of IgM
 - Secondary infections
 - High background antibody prevalence in some populations

Serological tests for arbovirus infection

- Enzyme immunoassays
 - IgG
 - IgM: sandwich and capture
 - Epitope-blocking
- Immunofluorescent antibody tests
 - IgG or IgM
- Haemagglutination inhibition
- Complement fixation titres
- Neutralisation titres

Haemagglutination inhibition



- In the presence of antibody that binds haemagglutinin, red cell haemagglutination is prevented
- Detects all antibody classes but does not distinguish between them
- Relatively insensitive

EASY

Alphaviruses

Diagnosis of alphavirus infection

- Virus detection not useful
- Serological tests

HI: measures total antibody IgG and IgM, but not very sensitive for IgM. Can show rise in titre

IFA IgM: Very sensitive for IgM. False positives rare.

EIA IgG: Good test for IgG, but cannot show rise in titre

EIA IgM: Sensitive for IgM. Some false positive results.

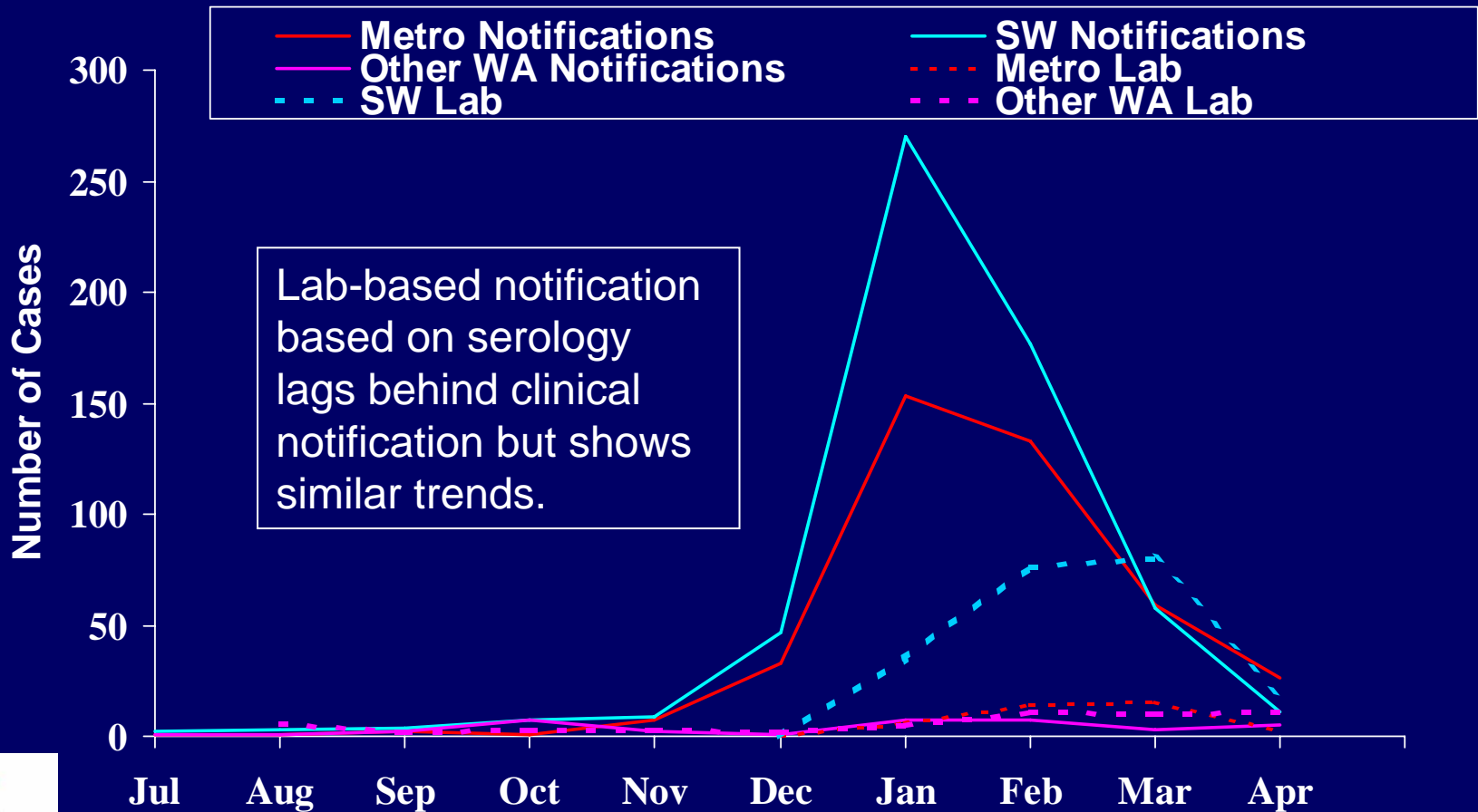
The tricky bits

- IgM routinely persists for months to years after infection and does not, in itself prove recent infection
 - Diagnosis of recent infection depends on showing IgG seroconversion or a rise in IgG
 - Otherwise it relies on the detection of IgM being consistent with the clinical and exposure history
- EIA (and occasionally IFA) can give false positive IgM
 - Where IgM is detected in the absence of IgG, then it is important to demonstrate IgG seroconversion on a convalescent sample
 - Occasional late IgM responses

RRV-EIA results

	Acute	Convalescent	
RRV-IgG	Neg	Pos	Acute RRV
RRV-IgM	Pos	Pos	
RRV-IgG	Neg	Neg	False positive IgM
RRV-IgM	Pos	Pos	
RRV-IgG	Pos	Pos	Recent, or recent past RRV
RRV-IgM	Pos	Pos	

Comparison of laboratory-based and clinical notifications of RRV Cases Jul 95 to Jun 96



Detection of Dual IgM

- Usually RRV and BFV positive. More common with EIAs
 - ? Cross-reaction
 - ? Recent dual infection
 - ? Recent past infection with one, recent infection with the other
- Collect convalescent sample to see if IgG rises to one/both.
- Examine exposure history, current virus activity.

Dual RRV and BFV IgM

		Acute	Conv	
HI/IFA IgM	RRV-HI	80	640	Acute RRV Past BFV
	RRV-IgM	Pos	Pos	
	BFV-HI	40	80	
	BFV-IgM	Pos	Pos	
EIA	RRV-IgG	Pos	Pos	Acute BFV and acute, recent or recent past RRV
	RRV-IgM	Pos	Pos	
	BFV-IgG	Neg	Pos	
	BFV-IgM	Pos	Pos	
EIA	RRV-IgG	Pos	Pos	Acute, recent or recent past RRV and BFV OR acute, recent or recent past RRV with false BFV IgM OR acute, recent or recent past BFV with false RRV IgM
	RRV-IgM	Pos	Pos	
	BFV-IgG	Pos	Pos	
	BFV-IgM	Pos	Pos	

Interpretation of alphavirus serology

- HI/IFA IgM

HI high, IgM positive: recent or recent past infection.
Follow up unlikely to show further rise

HI moderate, IgM positive: recent or recent past infection.
Repeat sample will probably show rise in antibody if recent.

HI low, IgM positive: very likely recent infection

- EIA

IgG positive, IgM positive: recent or recent past infection.

IgG negative, IgM positive: possible recent infection or false positive IgM. Repeat sample to show IgG seroconversion and confirm recent infection

Other markers of recent RRV infection

- IgA detection
 - IgA does not persist as long as IgM following recent infection
- IgG avidity
 - IgG following recent infection is low avidity

Using IgG avidity for diagnosis

- Patient serum added to two wells on an EIA plate
- One well is treated with a strong urea solution
- Antibody index is ratio of optical density after urea treatment to that without urea treatment
- Has been used for distinguishing between recent and past infection (e.g. rubella) and between primary infection and reactivation (e.g CMV)

Sindbis Virus

- Found in all mainland Australian states
- Mosquito isolations common, human infection uncommon
- Causes RRV-like disease, ? vesicular rash more common
- No commercial assays, use in house HI/IgM
- Antibody may cross-react with RRV/BFV, therefore if SIN serology positive, test for RRV/BFV to exclude cross-reaction
- May give false positive RRV/BFV serology using commercial EIAs

Chikungunya Virus

- Relatively common in southern and south-eastern Asia in 1960s, activity decreased in 1970s
- Emerged in Indonesia in 1982
- Re-emerged in Thailand in 1995, and Malaysia in 1998-99.
- Outbreaks in Reunion, Mauritius and India currently

Chikungunya in Klang

- Large outbreak in Klang, near Kuala Lumpur
- Clinical:
 - Fever (100%), myalgia (50%), arthralgia/arthritis usually mixed large and small joints (80%), backache (50%), rash (50%), headache (50%).
 - Illness persisted >6months in some patients
- Clinically identical to Ross River virus

Chikungunya diagnosis: The Klang experience

- Serological responses similar to that seen with other alphaviruses
- Those with elevated HI titres to CHIK commonly also had HI titres to Sindbis and some also had RRV HI. Possible cross-reaction or past SIN infection.
- IFA-IgM specific, i.e none of the IgM positive cases were positive for IgM to RRV, BFV, SIN

Serological diagnosis of chikungunya virus infection

- No commercial assays, use in house EIA/HI/IgM
- Antibody may cross-react with RRV/BFV
- May get weak false positive RRV/BFV serology, including IgM, using commercial EIAs
- Who to test
 - Returned from area of CHIK activity with RRV-like illness

Alphavirus serology: Take home

- Detection of alphavirus IgM is a reliable indicator of recent infection provided there has been a consistent clinical illness following potential exposure, and the infection was acquired in Australia
- Check for rising IgG whenever reasonable, especially if IgM without IgG, atypical illness, and/or unlikely recent exposure
- Consider other alphaviruses if infection acquired overseas

HARD Flaviviruses

NORTHERN TERRITORY NEWS

Photo 8954 9900 CLASSIFIED 9048 9999 DARWIN: Monday, July 24, 2000 88c (Includes Friday's) incl. GST

DIGGERS IN TIMOR
& THE GIRLS THEY LEFT BEHIND

TV SPECIAL INSIDE

Mosquito bite did this to him

Steven battles 'nasty virus'

By SUELLEN HINDE

A common mosquito bite did this.

Steven Dow, 26, lies paralysed in Royal Darwin Hospital after being infected with the potentially fatal mosquito-borne Murray River encephalitis. He can only move his eyes.

The English backpacker has been a patient in intensive care for almost three months after collapsing at Kakadu National Park in late April. He was at the end of a 15-month tour of Australia.

It is believed he contracted the mosquito-

Continued Page 2

Complicated and horrifying ... British backpacker Steven Dow in Royal Darwin Hospital yesterday. Pictures: DAWN GAWLIK

Diagnosing flavivirus infections in Australia

- Compared with alphaviruses
 - Greater emphasis on accurate diagnosis because of greater personal and public health significance
 - Serology is more difficult to interpret
 - Direct detection methods have greater role in helping to confirm infection and the identify of the infecting virus

Serological diagnosis of flavivirus infections

- Flavivirus IgG is broadly cross-reactive, i.e. most tests for IgG will not tell you which virus caused the infection
- Flavivirus IgM is less cross –reactive and may provide some indication of the infecting virus, provided that reactivity is restricted to a single virus
- Detection of IgM may not always indicate recent infection

Flavivirus serology

	Acute	Convalescent
MVEV-HI	80	640
MVEV-IgM	Pos	Pos
KUN-HI	40	160
KUN-IgM	Pos	Pos
JEV-HI	160	2560
JEV-IgM	Pos	Pos
DENV-HI	80	1280
DENV-IgM	Pos	Pos

IgM detection for flaviviruses

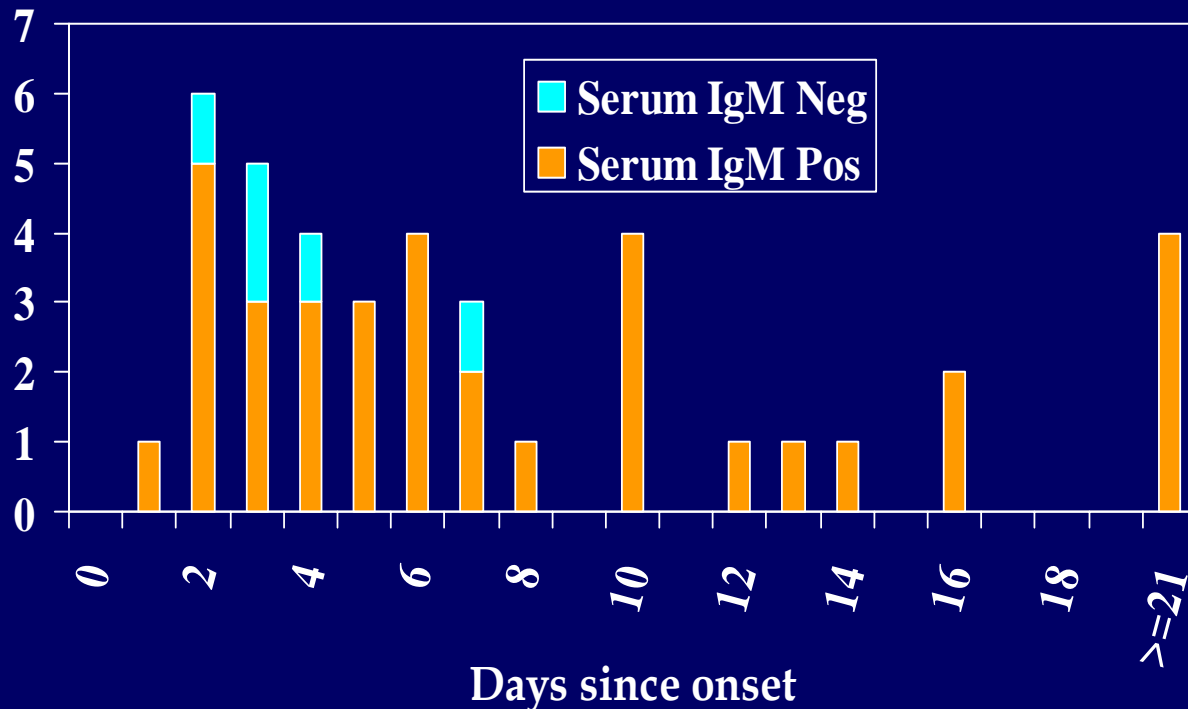
- Due to the antigenic similarity of the flaviviruses, antibody cross-reactions are common and may be broad
- IgM cross-reactivity is less than IgG, but will vary with the test used and the virus
- A positive IgM is most reliable if there is only one possible infecting flavivirus or where it is shown that there is no IgM to other flaviviruses
- Absence of IgM makes recent infection with that virus unlikely, but does not completely exclude it.

IgM for diagnosis of MVE/KUN encephalitis

- IgM detection in serum
 - 28 patients positive
 - 2 patients negative
- IgM detection in CSF
 - 14 patients positive
 - 4 patients negative
 - samples collected days 1,1,3 and 5 after onset

MVE/KUN encephalitis 1987-2000

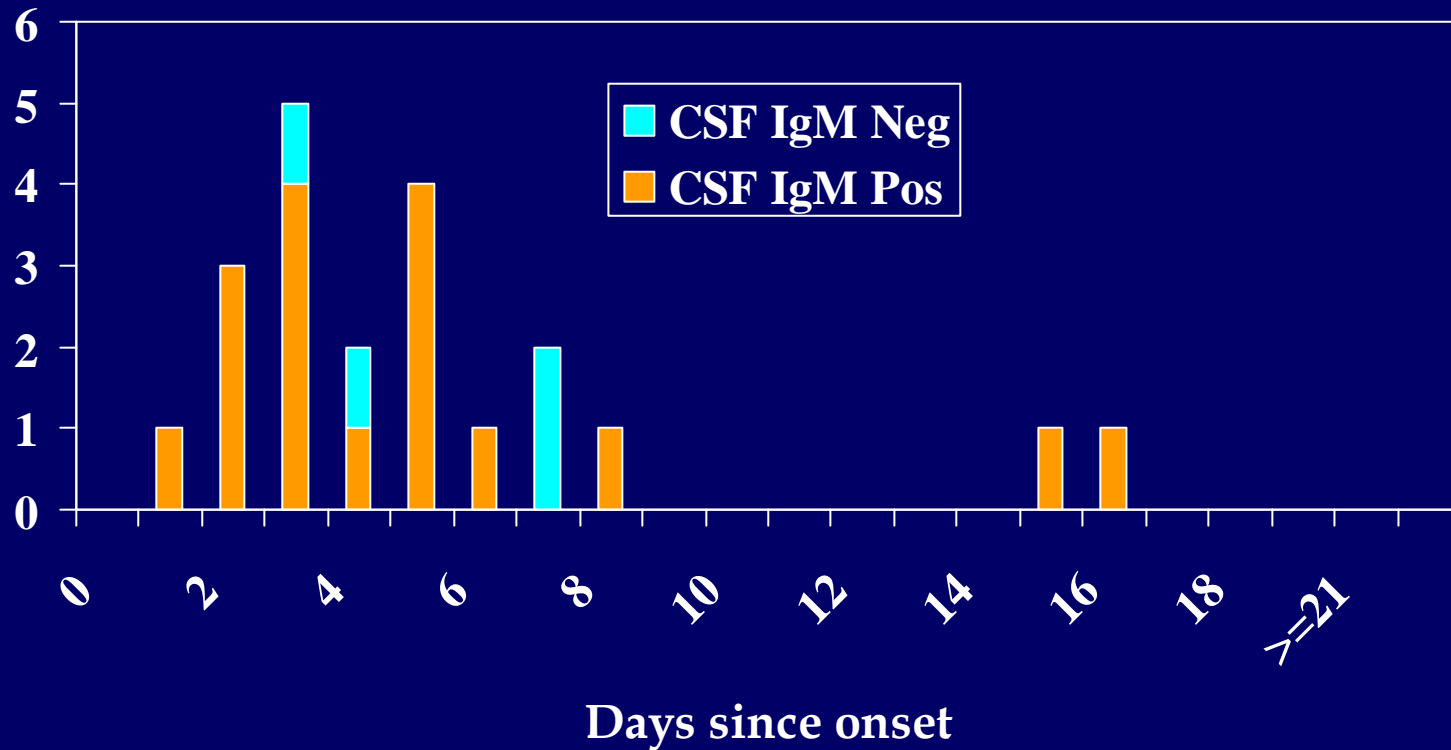
Serum IgM by IFA



Positive IgM detected in one case 150 days after onset

MVE/KUN encephalitis 1987-2000

CSF IgM by IFA



IgM in Japanese encephalitis

- IgM is present in serum of 75%-90% of cases within a few days of onset of illness, and nearly all by 10 days after onset.
- IgM detection in CSF using sensitive methods like antibody-capture EIA may be more sensitive than detection in serum
- A recent study of samples from Thai patients
 - IgM found in 60% of serum and 90% of CSF samples collected 1-4 days after onset of illness.
 - All CSF samples positive by day 7, 100% positive results in serum not until day 13.

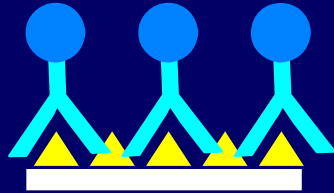
Serological diagnosis of flavivirus infections: the pitfalls

- Rise in IgG
 - Cross reactive IgG
 - Immune recall (original antigenic sin)
- Detection of IgM
 - False positive IgM
 - Cross reactive IgM
 - Persistent IgM
 - Lack of IgM in secondary infections
 - Late IgM in some patients, especially seriously ill

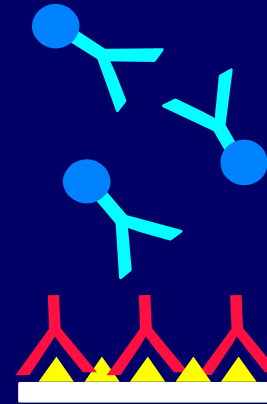
Specific tests for flavivirus IgG

- Neutralisation tests
 - Relatively specific provided a suitable range of flaviviruses is tested and there is a significantly higher titre to one flavivirus
- Epitope-blocking EIA
 - Measure ability of patient antibody to block binding of specific monoclonal antibodies

Epitope-blocking EIA for detection of flaviviruses-specific antibody



Patient lacks specific antibody and labelled McAb is able to bind



Patient has flavivirus antibody which blocks the epitope targeted by the McAb



Epitope-specific monoclonal antibody



Flavivirus antibody in patient serum



Solid phase coated with target virus

Can be used for detection of specific antibody to MVE, Kunjin, Japanese encephalitis and West Nile viruses

Epitope-blocking EIA for differentiation between antibody to MVE and KUN

		Percent inhibition using		
		3H6 (Flavi)	10C6 (MVE)	3112G (KUN)
MVE	Early	56%	11%	<10%
	Late	51%	63%	<10%
KUN		96%	19%	94%
Mixed		95%	94%	81%

Epitope-blocking EIA

- Need to find a monoclonal antibody that will react with a specific epitope and will efficiently block cross-reactive antibody
- May not provide clear results with early antibody responses
- May not work where it is a second flavivirus infection, e.g. detection of JEV IgG when there has been previous MVEV infection

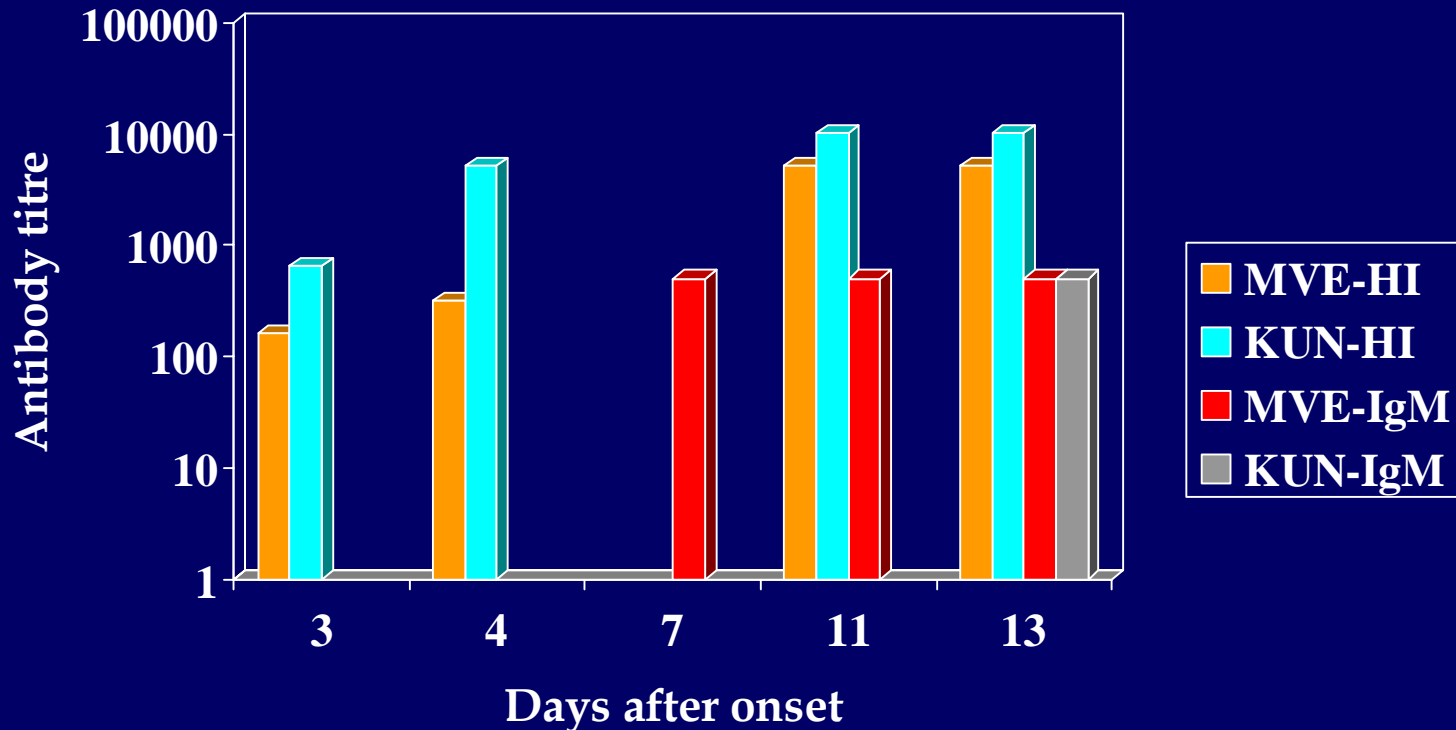
Neutralisation titres for flaviviruses

- Measures ability of patient's serum to prevent growth of a range of flaviviruses
- NT to the infecting virus is at least 4 fold higher than the NT to any other virus
- BUT
 - Some viruses grow poorly
 - NT can be very low, making it difficult to define a four-fold difference
 - Not always discriminatory

Original antigenic sin

- Occurs when the patient has had a previous infection with a closely related flavivirus
- When infected with the new flavivirus, their initial antibody response is to the previously infecting virus
- Examples: Secondary dengue, MVE infection following KUN

Misleading HI and IgM results: 4yo male. PCR positive for MVE



Past KUN infection

KUN HI rose first. Late appearance of IgM.

Blocking EIA showed both MVE and KUN antibody

Diagnosing dengue: Primary versus secondary infection

- Primary infection
 - IgM detected
 - Rise in IgG
- Secondary infection
 - Variable IgM response
 - Rapid rise in IgG
 - Initial IgG may be directed to the previously infecting serotype, therefore even neutralisation may be misleading

Serological diagnosis of flavivirus infections: the pitfalls

- Rise in IgG
 - Cross reactive IgG
 - Immune recall (original antigenic sin)
- Detection of IgM
 - Cross reactive IgM
 - Persistent IgM
 - Lack of IgM in secondary infections
 - Late IgM in some patients, especially seriously ill

Persistence of IgM following flavivirus infection

- West Nile
 - 50% still positive 2 months after onset
- MVE encephalitis
 - IgM usually persists throughout the follow-up period. Three patients followed to 4, 5 and 6 months after onset of illness all remained IgM positive in serum

Defining recent infection: IgG avidity for dengue diagnosis

IgG EIA. Optical density with and without treatment with 8M urea for 5 mins or 7M urea for 10mins

	Primary infection No (%)	Secondary infection No (%)
Avidity index ≤ 24	27 (100%)	2 (6.7%)
Avidity index > 24	0	28 (93.3%)

Nucleic acid detection for flavivirus diagnosis

- Limited availability,
- Many patients seen too late for it to be useful
- Useful for
 - early diagnosis,
 - definitive identification and typing of virus,
 - resolving tricky serology
 - molecular epidemiology

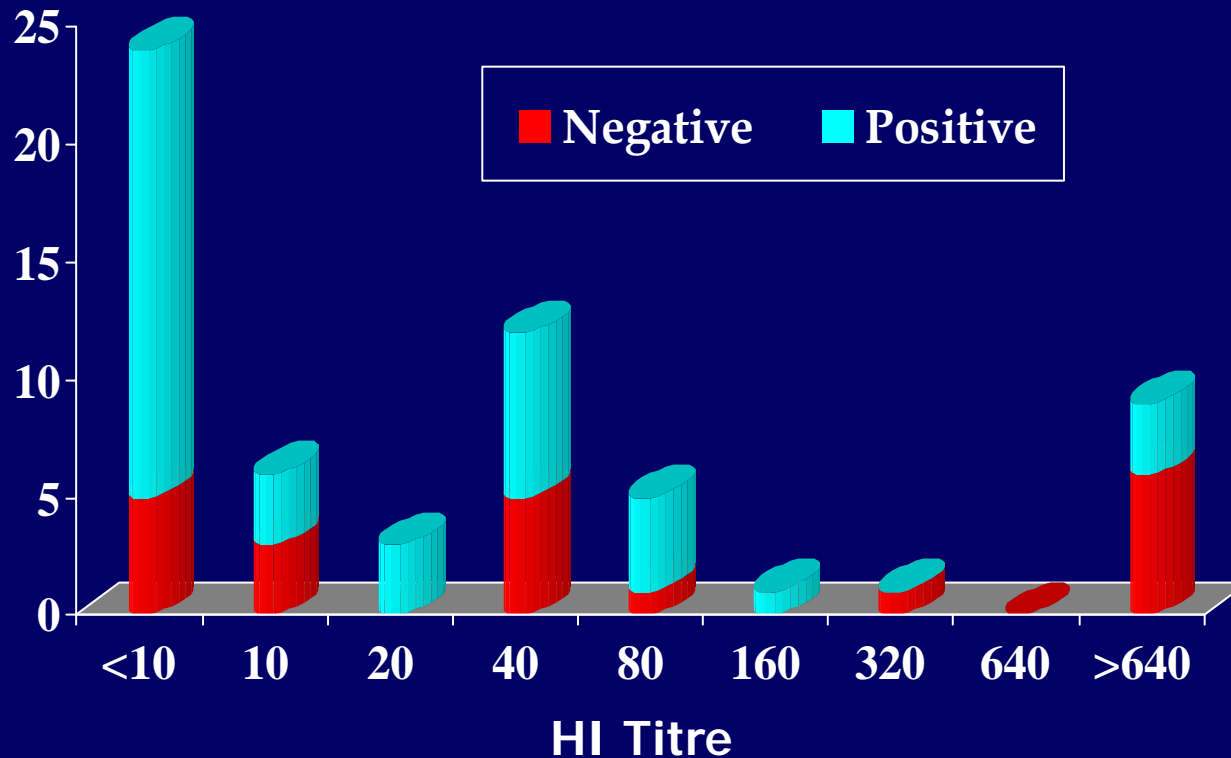
PCR for detection of WNV in CSF

	CSF		Serum	
	TaqMan	RT-PCR	TaqMan	RT-PCR
Seropositive	16/28	0/28	4/28	0/28
Seronegative	30/30	0/42	0/19	ND

Lanciotti RS. J. Clin. Microbiol. 2000. **38**: 4066-4071

Dengue PCR versus HI titre

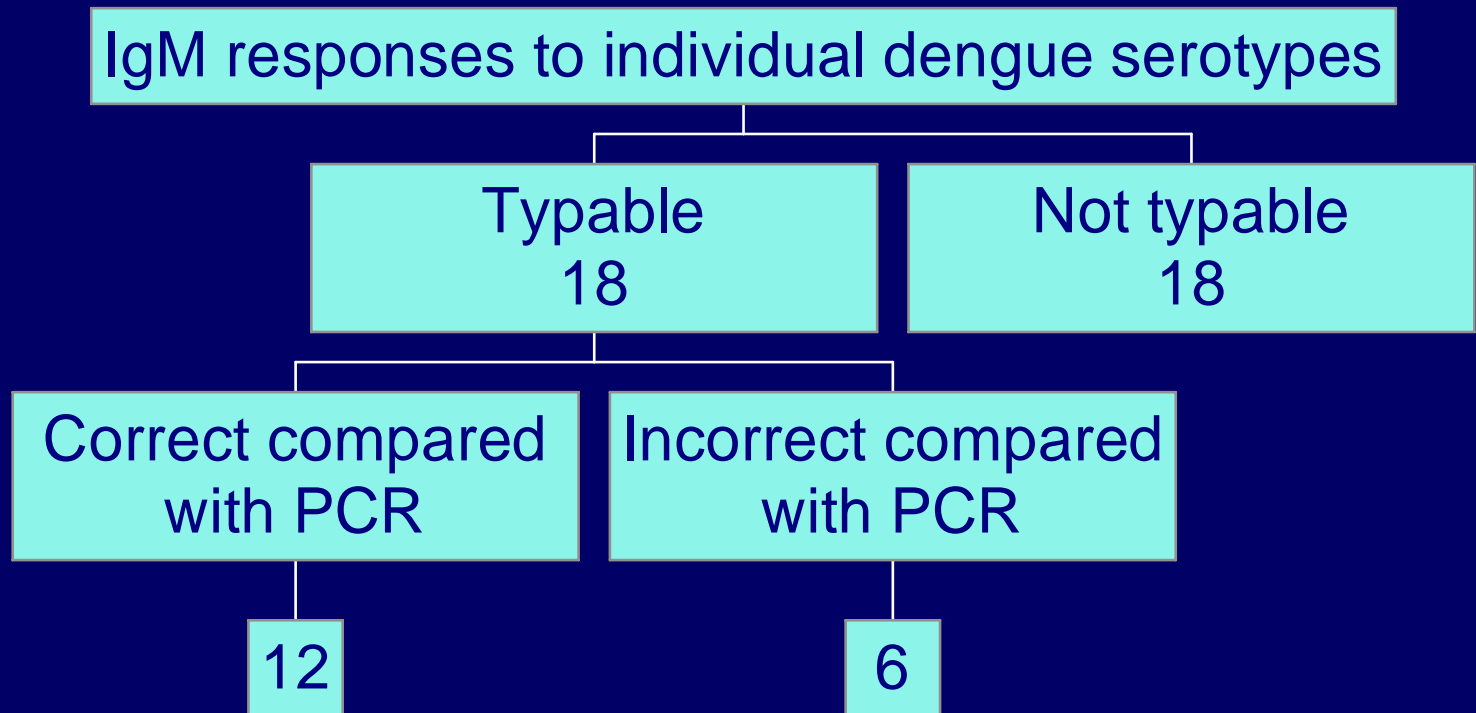
Nested PCR directed to the envelope gene



Dengue RNA detected in 20/25 IgM positive serum samples,
and 12/14 IgM negative samples

Serotype specificity of Dengue IgM

Indirect IFA using 4 separate dengue types. Type assigned serologically if there was IgM reactivity to one type only, or where the reactivity was substantially stronger to a single serotype.



Diagnosis of West Nile virus infection

- Kunjin is a strain of WNV
- Patients with WNV infection have antibody that is serologically indistinguishable from Kunjin
- Confirmation of WNV infection requires sequencing of isolate or PCR product
- Suspected where patient has serological evidence of KUNV infection, but history of exposure to WNV overseas

Flavivirus infections: Take home

- Usually reliably achieved by serological methods
- IgM detection is usually reliable, provided there is a good clinical and exposure history and the IgM reactivity is strong. It is most reliable if only one flavivirus is likely.
- Confirm IgM by showing a rise in IgG wherever possible, particularly for severe illnesses and for arboviruses of special public health importance
- Consider exotic arboviruses in returned travellers or overseas residents