蟲媒傳染病之血清學檢驗

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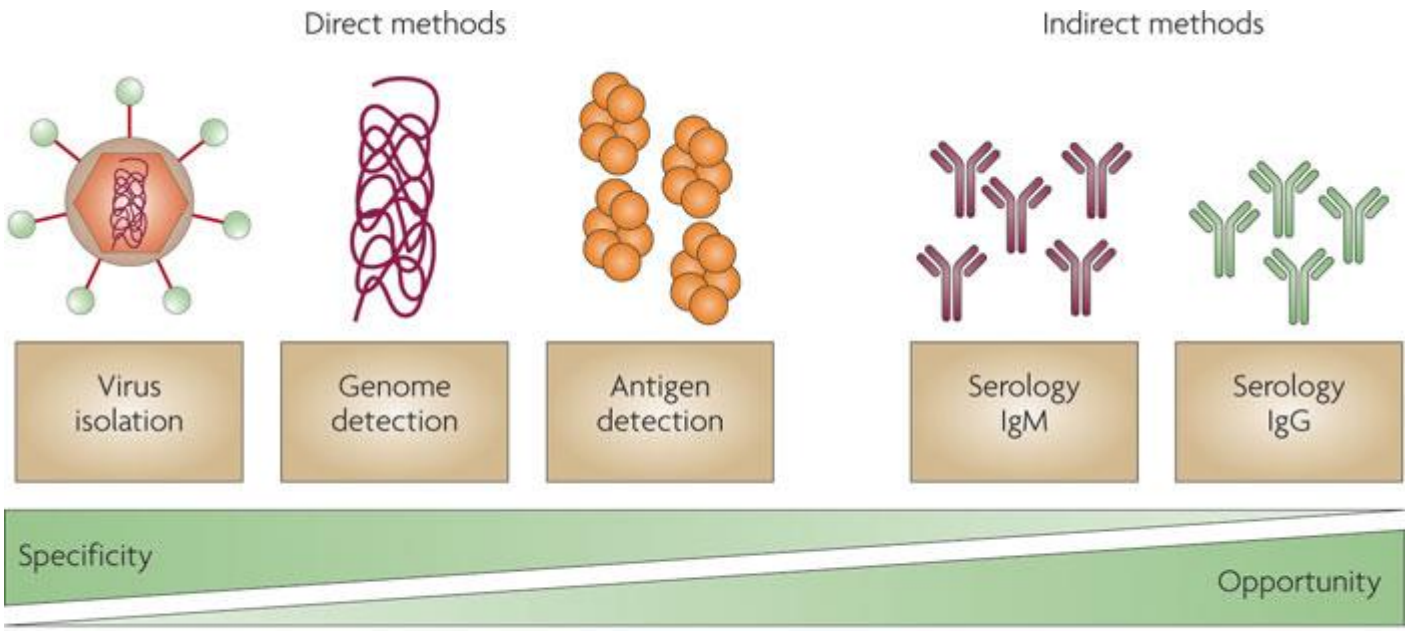

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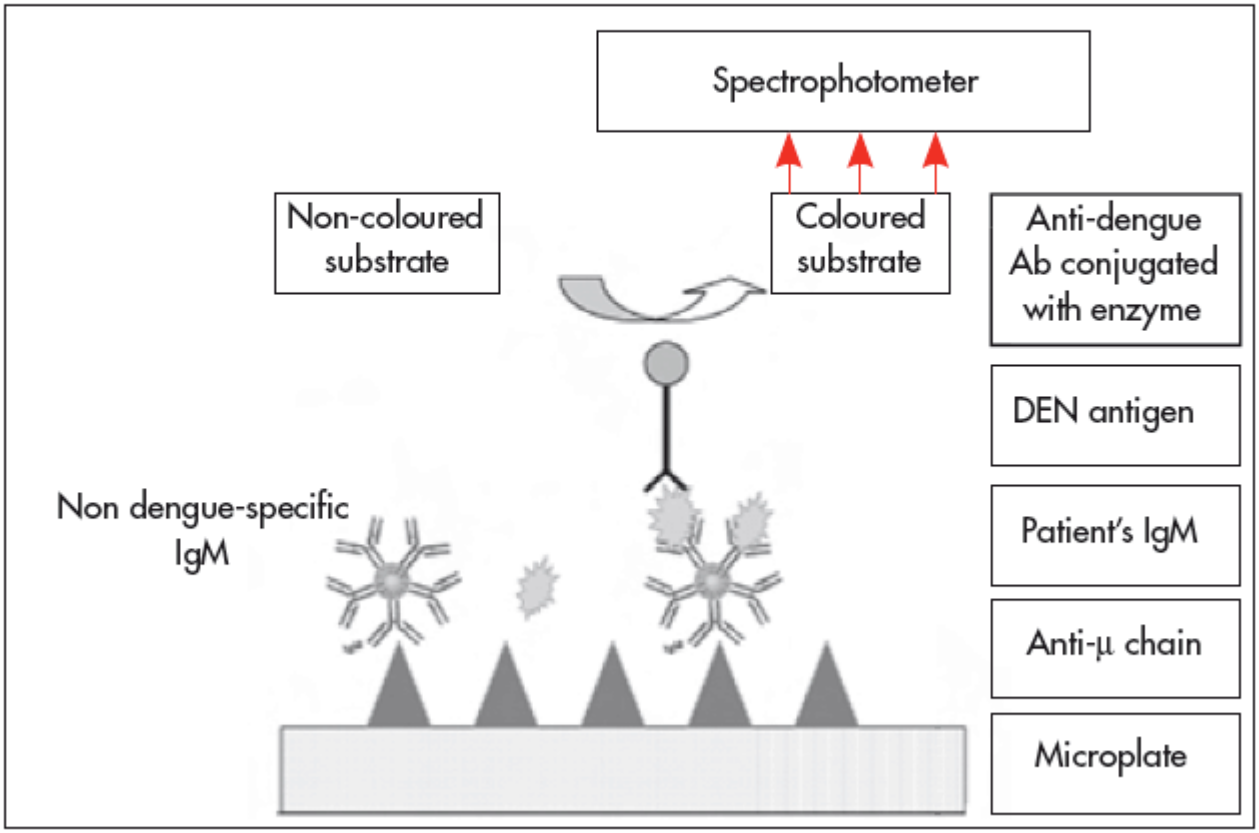
Diagnostic tests	Advantages	Limitations
Viral isolation and identification	• Confirmed infection • Specific • Identifies serotypes	• Requires acute sample (0–5 days post onset) • Requires expertise and appropriate facilities • Takes more than 1 week • Does not differentiate between primary and secondary infection • Expensive
RNA detection	• Confirmed infection • Sensitive and specific • Identifies serotype and genotype • Results in 24–48 hours	• Potential false-positives owing to contamination • Requires acute sample (0–5 days post onset) • Requires expertise and expensive laboratory equipment • Does not differentiate between primary and secondary infection
Antigen detection		
Clinical specimens (for example, using blood in an NS1 assay)	• Confirmed infection • Easy to perform • Less expensive than virus isolation or RNA detection	• Not as sensitive as virus isolation or RNA detection
Tissues from fatal cases (for immunohisto-chemistry, for example)	• Confirmed infection	• Not as sensitive as virus isolation or RNA detection • Requires expertise in pathology
Serological tests		
IgM or IgG seroconversion	• Confirmed infection • Least expensive • Easy to perform	• IgM levels can be low in secondary infections • Confirmation requires two or more serum samples • Can differentiate between primary and secondary infection*
IgM detection (single sample)	• Identifies probable dengue cases • Useful for surveillance, tracking outbreaks and monitoring effectiveness of interventions	• IgM levels can be low in secondary infections

*Primary infection: IgM-positive and IgG-negative (if samples are taken before day 8–10); secondary infection: IgG should be higher than 1,280 haemagglutination inhibition in convalescent serum.

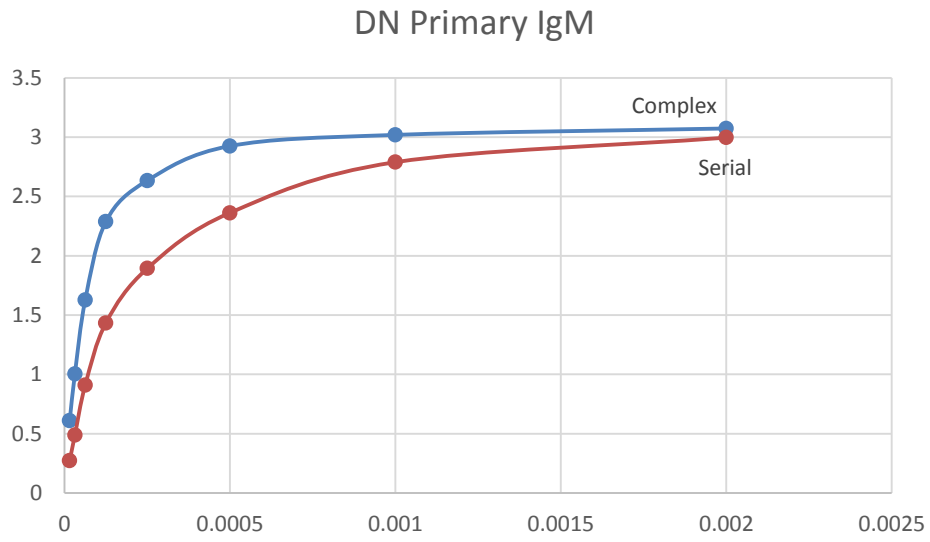
Principle of a MAC-ELISA test

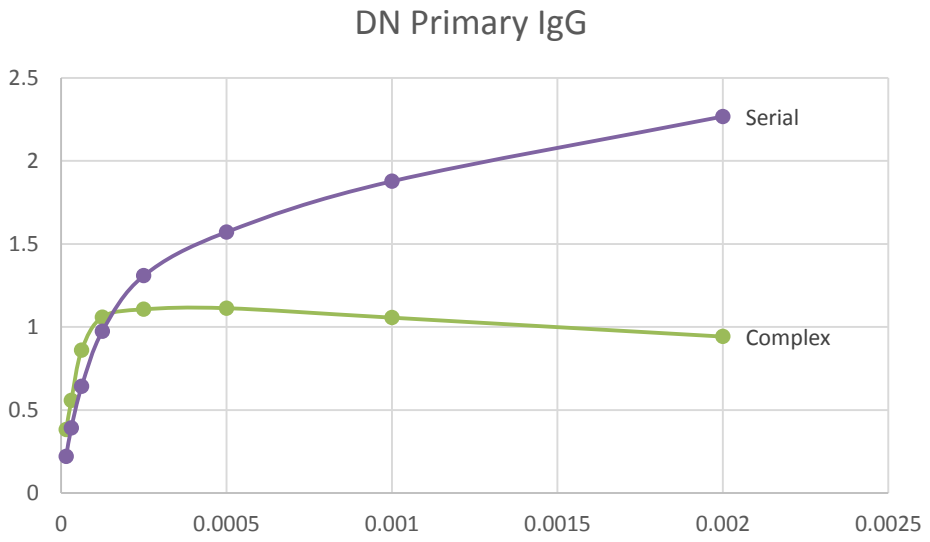


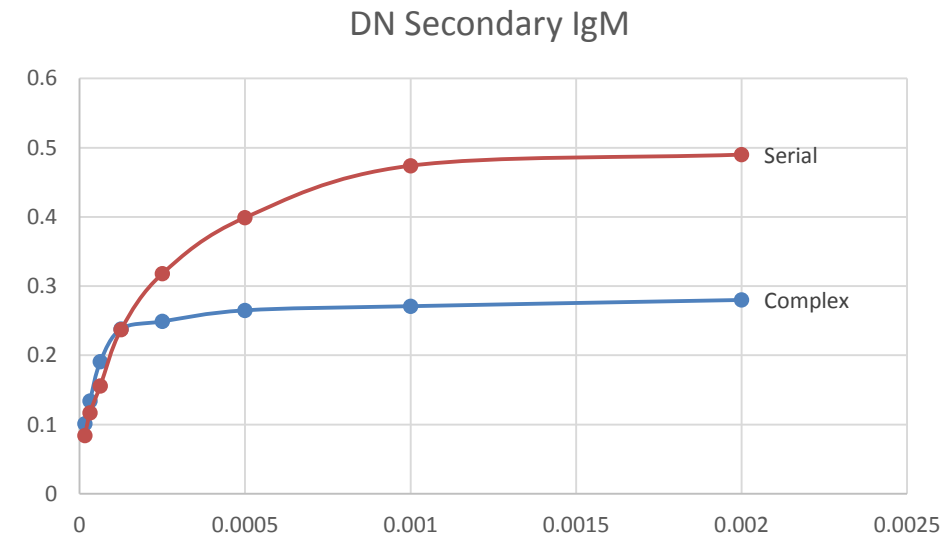
Standard Protocol of MAC-ELISA employs anti-human IgM antibody absorbed polystyrene plate to capture human IgM in patients' serum, and add antigen, tracer (detector) antibodies in a step-by-step manner.

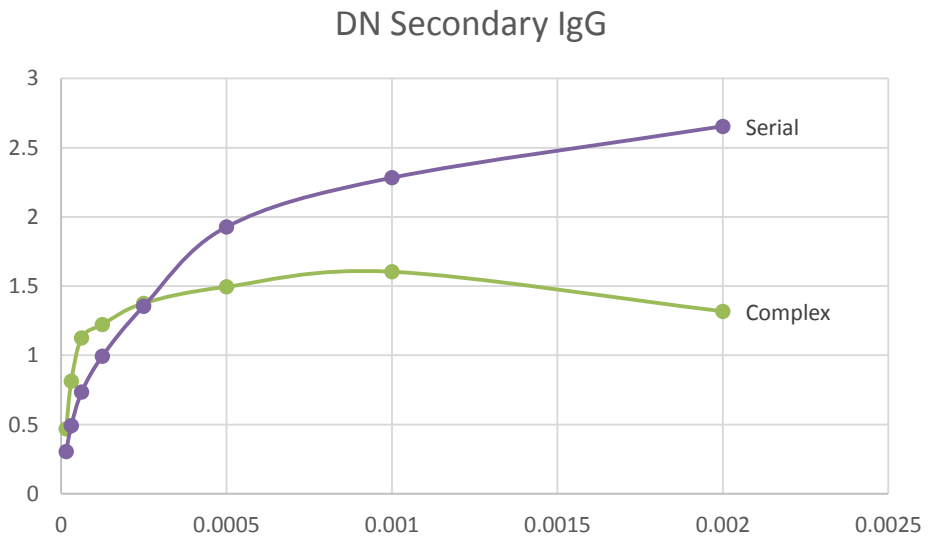
Our laboratory has developed a modified MAC-ELISA method 12 years ago, which simultaneously capture patients' serum IgM to anti-human IgM plates, and in an independent reaction vessel, mix tracer (detector) antibody and viral antigen together to form Ag-Ab precomplex. MAC-ELISA results can readily be obtained by adding the Ag-Ab precomplex to human IgM captured plates, followed by color development.

Binding Behavior of Virus-TracerAb precomplex is Different from that of Step-by-step addition of Virus and TracerAb (I)

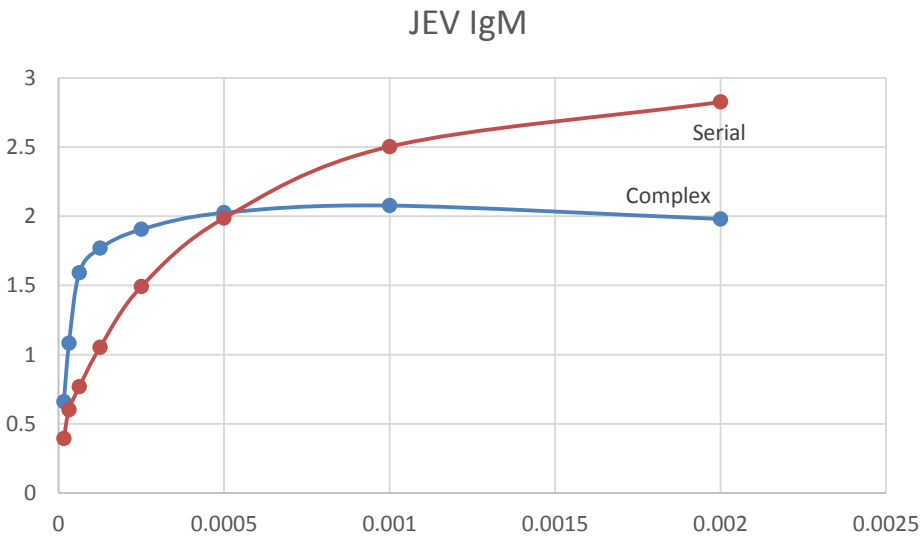


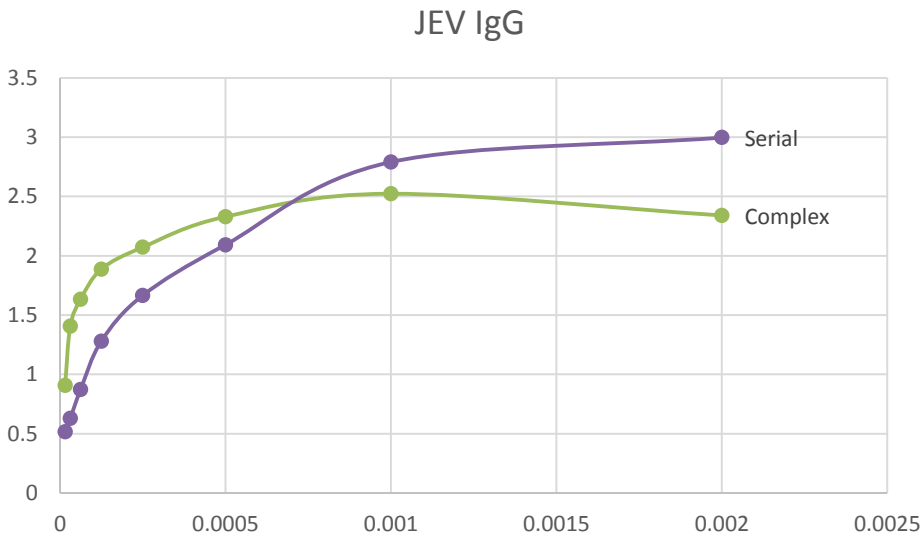


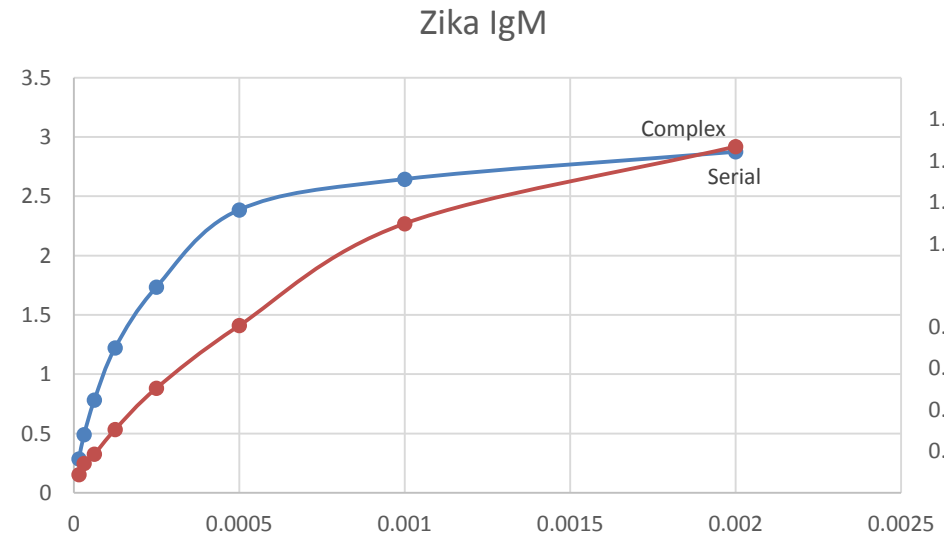


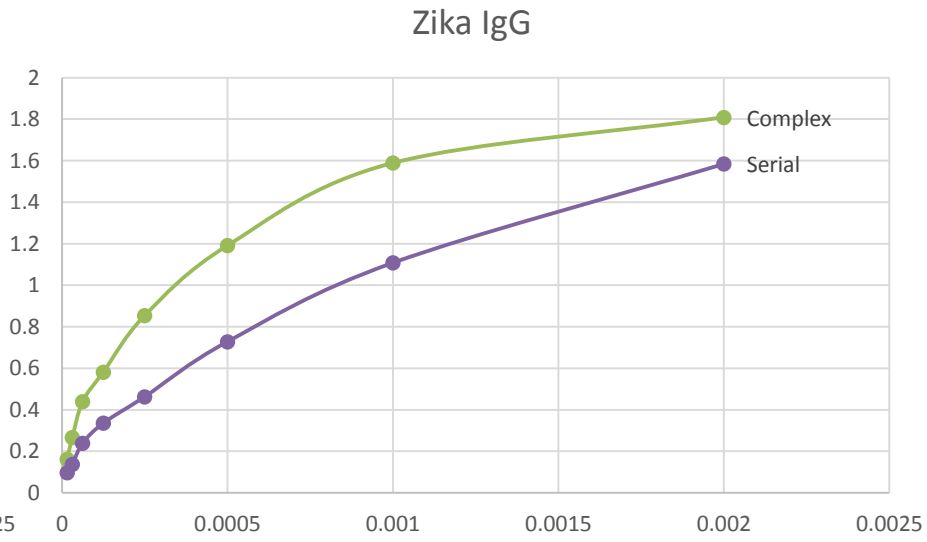


Binding Behavior of Virus-TracerAb precomplex is Different from that of Step-by-step addition of Virus and TracerAb (II)









Distinct Feature of Virus/TracerAb Precomplex Binding Behavior

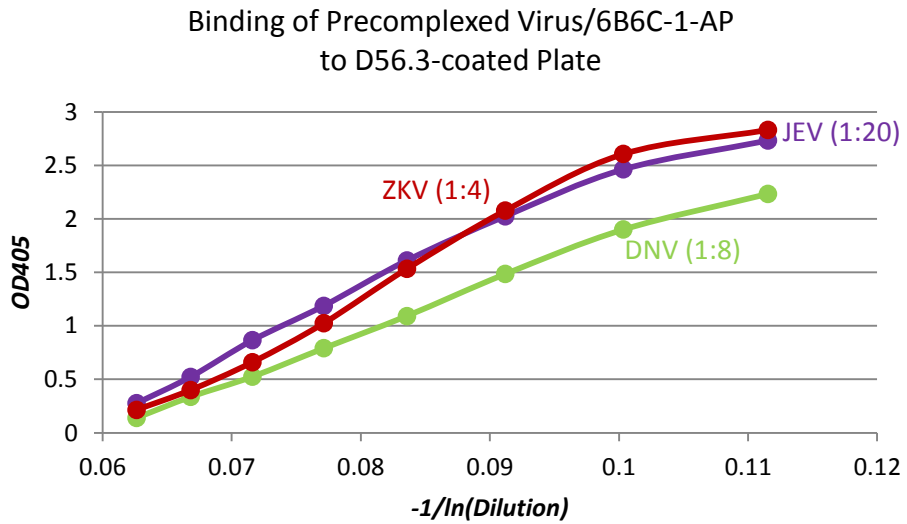
Rapid Saturation beyond a Given Concentration of TracerAb

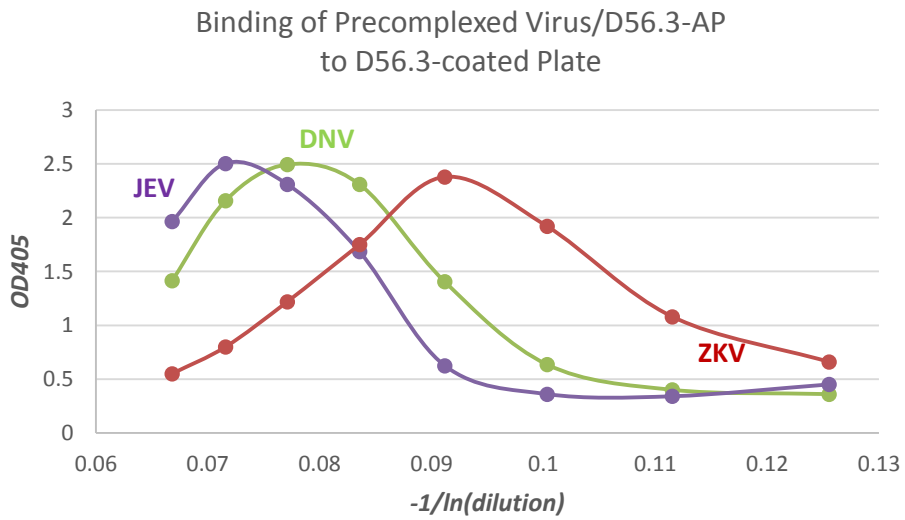
f (MAC-ELISA Specificity) =

f (Specific epitopes recognized)/f (Cross-reactive epitopes recognized)

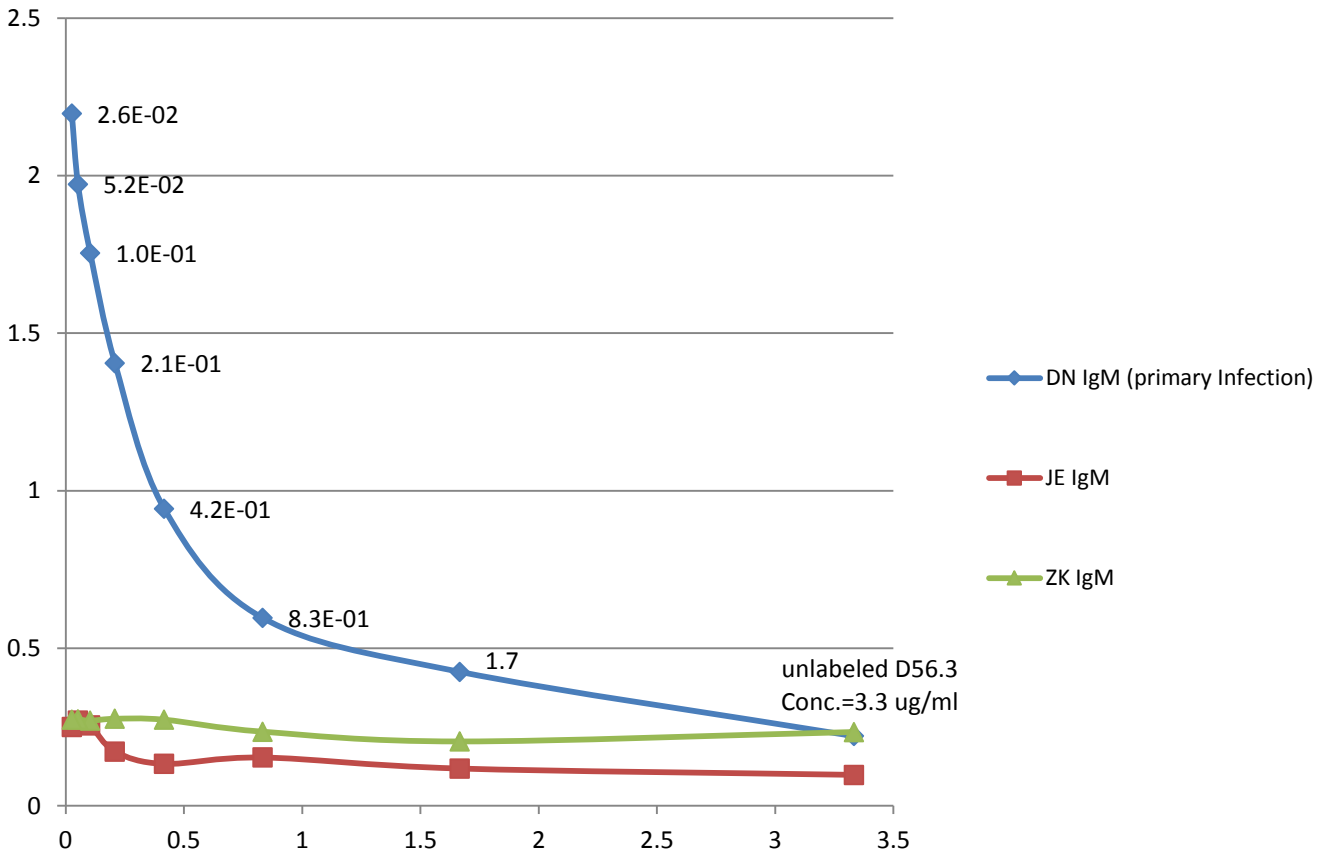
Could Virus/TracerAb precomplex interfere with Virus Binding to Cross-reactive Epitopes of Patients' serum IgM?

D56.3 and 6B6C-1 mAb Recognize Distinct Epitopes on DNV, JEV and ZKV

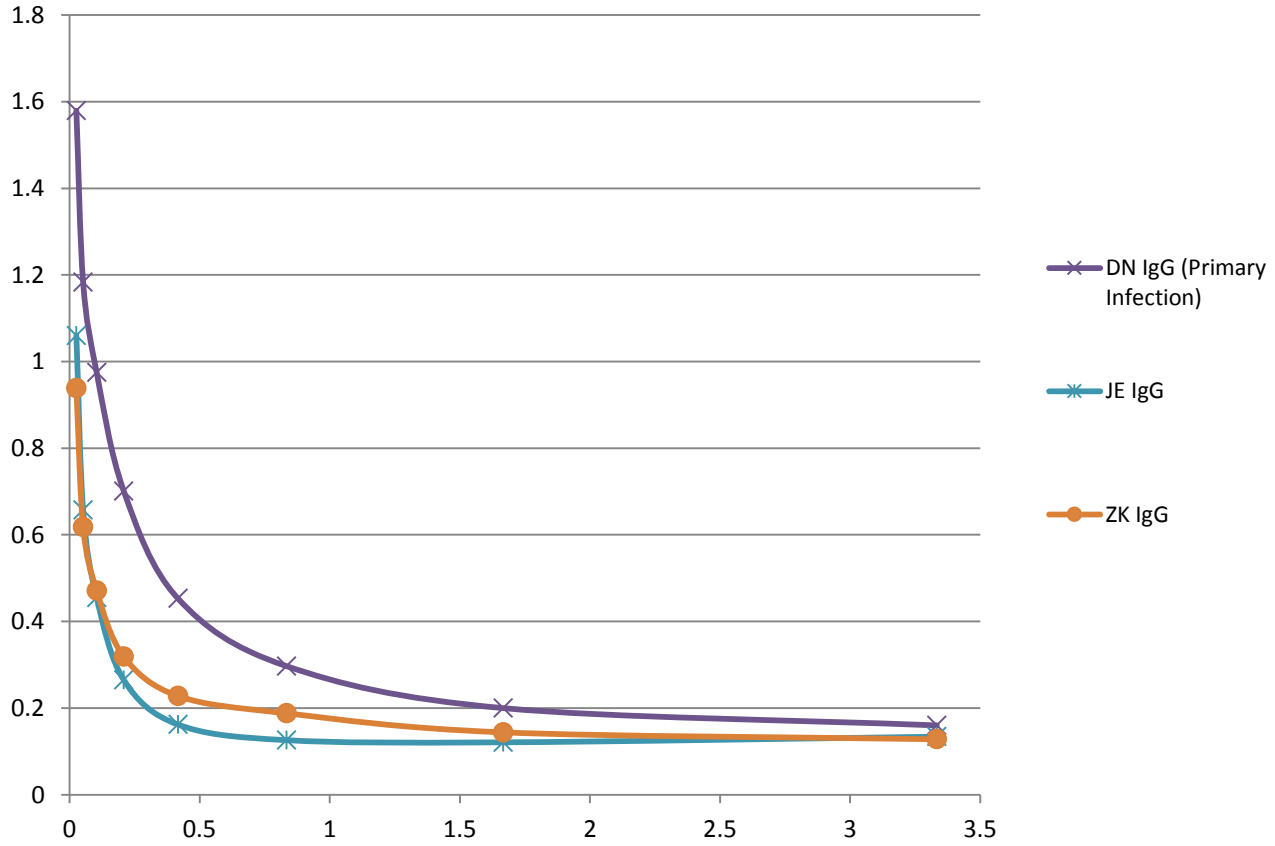




Competition Assay Demonstrated D56.3 Epitope is One of the Major Human IgM Epitope Induced after Primary Dengue Infection with Minor Cross Reactivity to Both JE and ZK



Competition Assay Demonstrated the Presence of D56.3 Epitope in DNV, JEV, or ZKV infected Patients' IgG



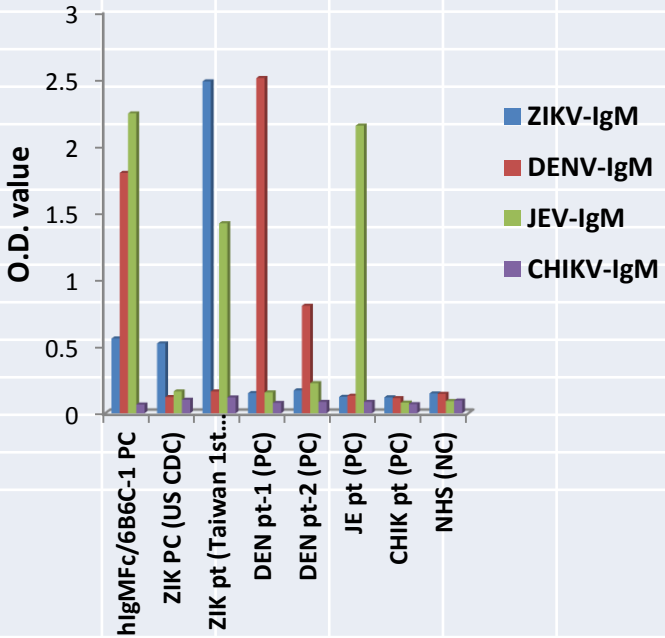
Multiplexed MAC-ELISA Allowed Simultaneous Detection of Dengue, JE, Zika, and Chikungunya Infections

	ZIKV-IgM	DENV-IgM	JEV-IgM	CHIKV-IgM
hIgMFc/6B6C-1 PC	0.559	1.796	2.24	0.065
ZIK PC (US CDC)	0.522	0.121	0.163	0.101
ZIK pt (Taiwan 1st case)	2.479	0.163	1.42	0.118
DEN pt-1 (PC)	0.15	2.504	0.156	0.077
DEN pt-2 (PC)	0.171	0.804	0.225	0.085
JE pt (PC)	0.123	0.131	2.149	0.085
CHIK pt (PC)	0.119	0.113	0.079	0.068
NHS (NC)	0.148	0.145	0.091	0.095

6B6C-1 Humanized chimeric IgM was given at 1:50 dilution

US CDC positive antiserum was given at 1:100 dilution

Virus dilutions were determined empirically where Dengue was given at dilution of 1:6, JE at 1:20, CKV at 1:12 and ZKV at 1:4



Conclusion and Future Prospects

a) D56.3 mAb might define one of the major IgM epitopes in Dengue infected patient, but not in JEV or ZKV infected ones.
b) Formation of Virus/TracerAb precomplex might be advantageous for MAC-ELISA assay by competitive blocking of cross-reactive epitopes, and thus, improving the assay specificity.
c) Our modified MAC-ELISA system should allow multiplexed serological detection of at least DNV, JEV, ZKV and CKV, and is of great potential for detection of other arboviruses.
d) Further blocking of cross-reactive epitopes is absolutely required for Arbovirus IgG ELISA assays to ensure reasonable specificity.