

Antibody Engineering at Fc Region to Diminish Antibody Dependent Enhancement of Cross-Neutralizing Human Monoclonal Antibody Against Dengue Virus

Asst. Dr. Pannamthip Pitaksajjakul

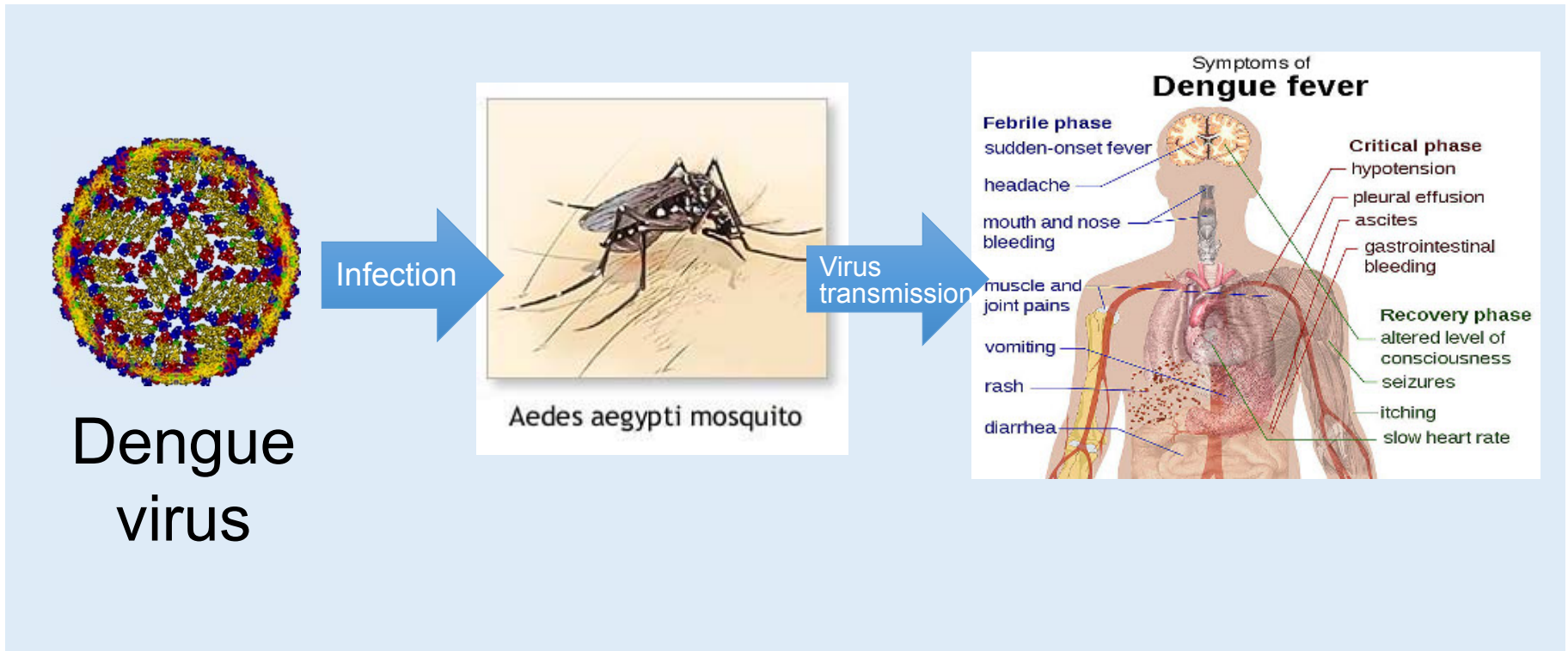
**Center of Excellence for Antibody Research
Faculty of Tropical Medicine
Mahidol University**

**Joint International Tropical Medicine Meeting
7 December 2017**

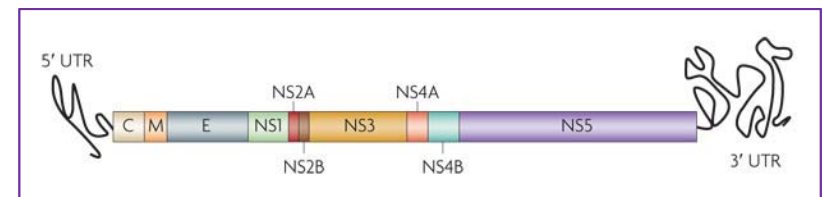


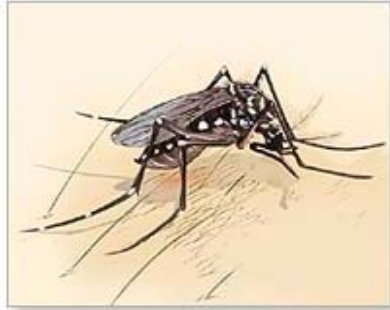
Dengue virus

Transmitted from mosquito to humans causing a fatal disease.

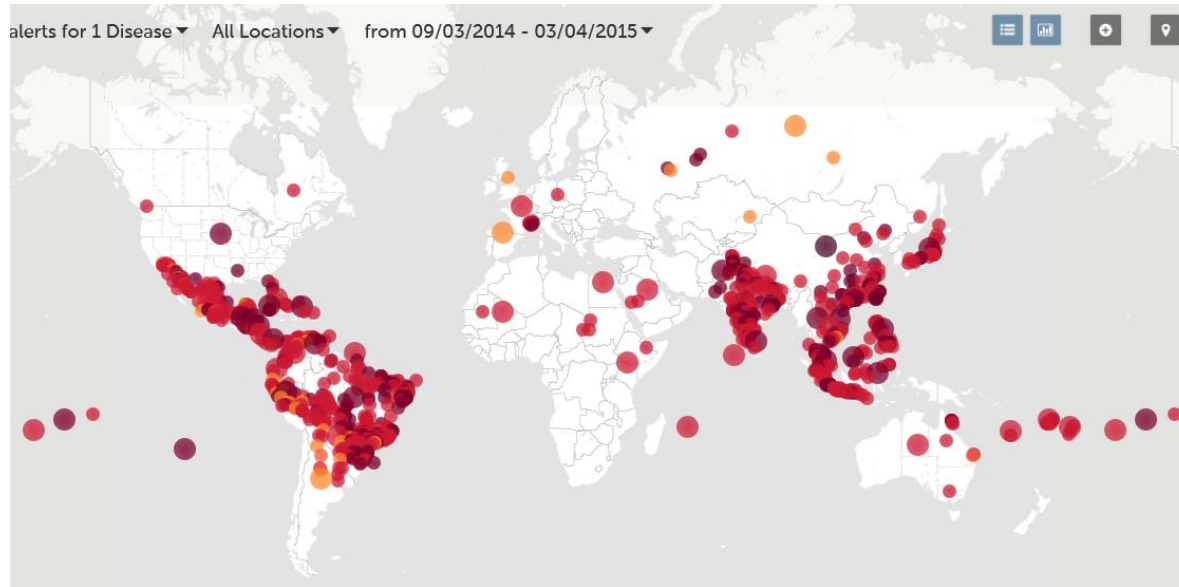


- ❖ Family *Flaviviridae*, genus *Flavivirus*
- ❖ Single stranded non-segmented RNA virus
- ❖ 4 distinct serotypes: DENV-1 to -4





Aedes aegypti mosquito



(<http://www.healthmap.org>)

40%, 100 millions are at risk all over the world/ year

20% develop to severe disease leading to death (22,000 death/year)

No specific drugs or treatment available





Production of therapeutic antibody

Preparing hybridoma cells

Screening HuMAbs

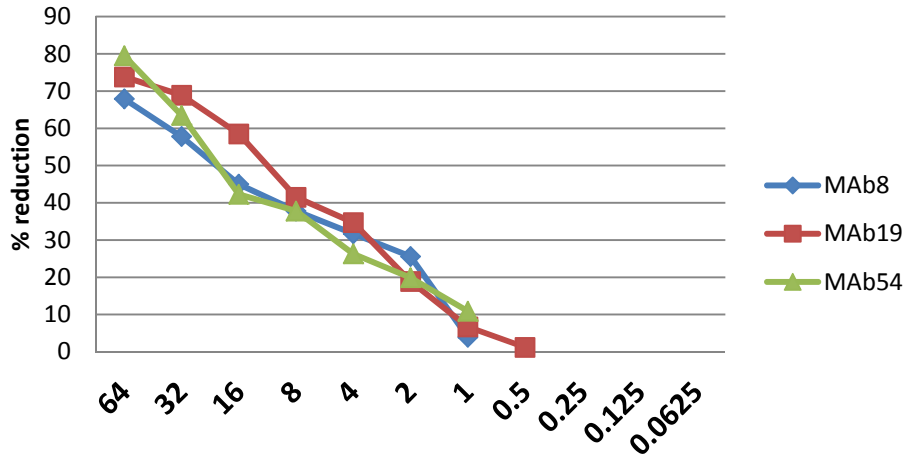
- ❖ **Immunofluorescence reactivity**
- ❖ **Viral Neutralization test**
- ❖ **Target protein detection**

Selection of HuMAbs

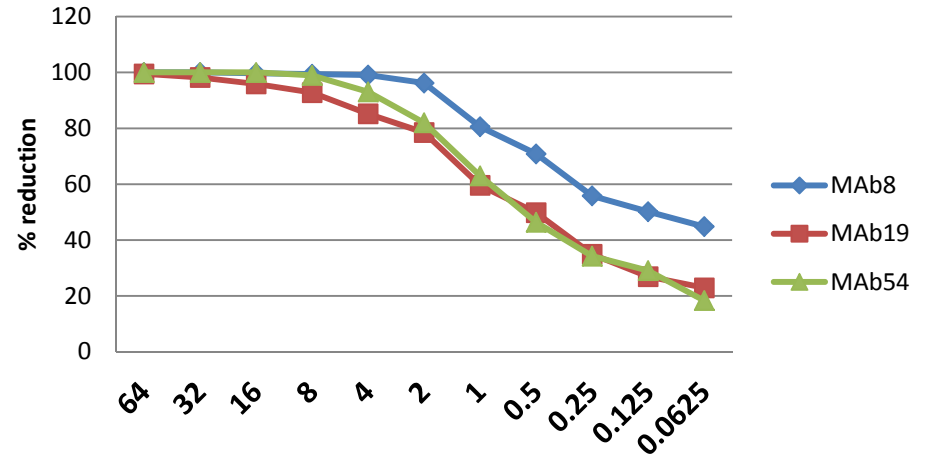
NT study with DENV Lab strains

(DENV1: Mochizuki, DENV2: NGC, DENV3: H87, DENV4: H241)

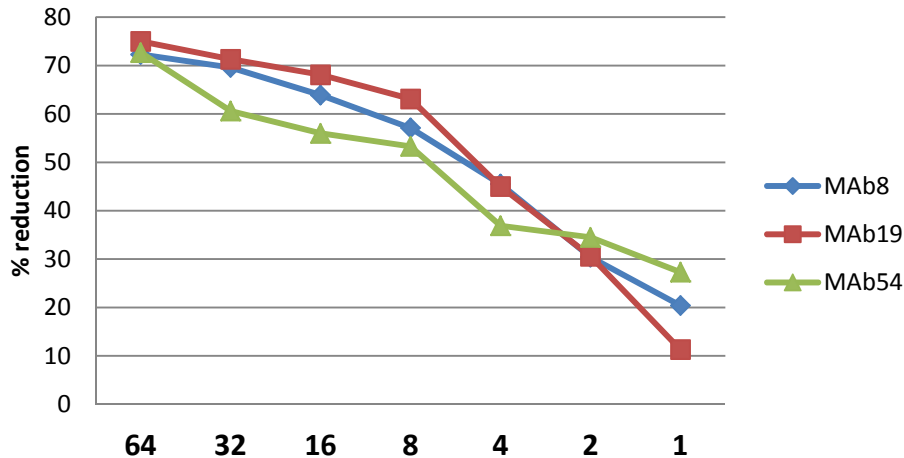
NT for DENV1



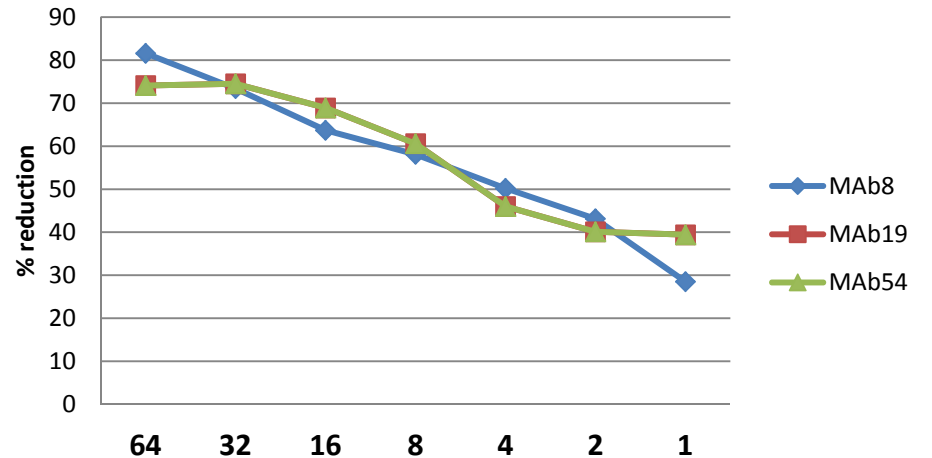
NT for DENV2



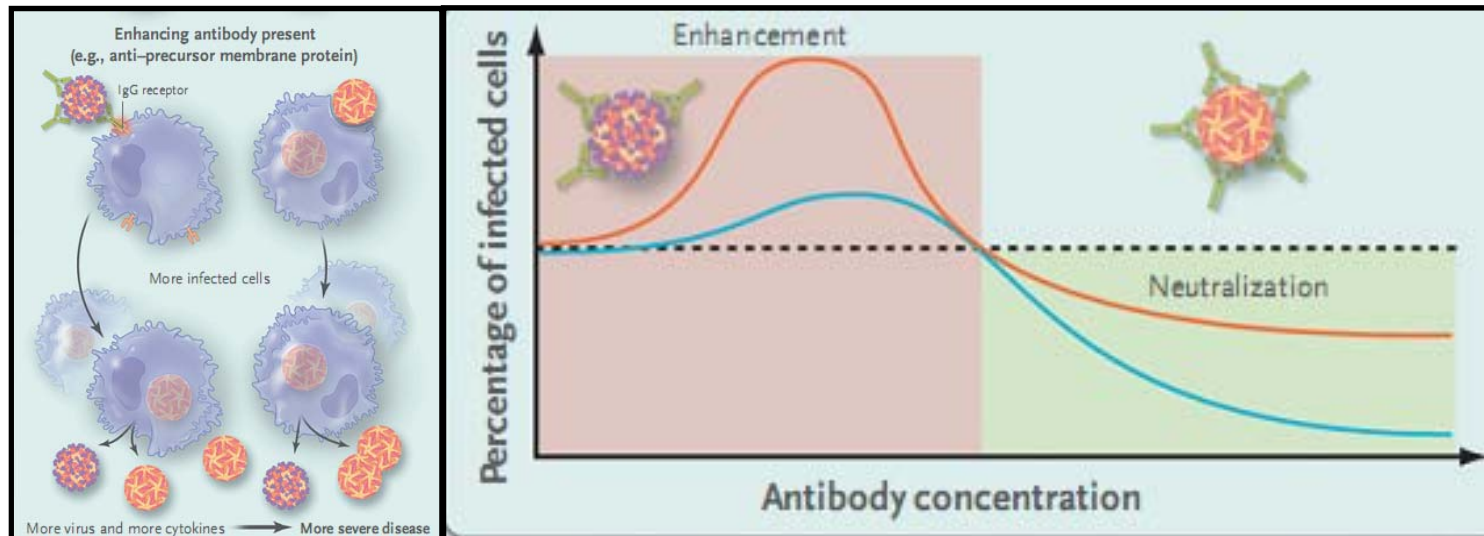
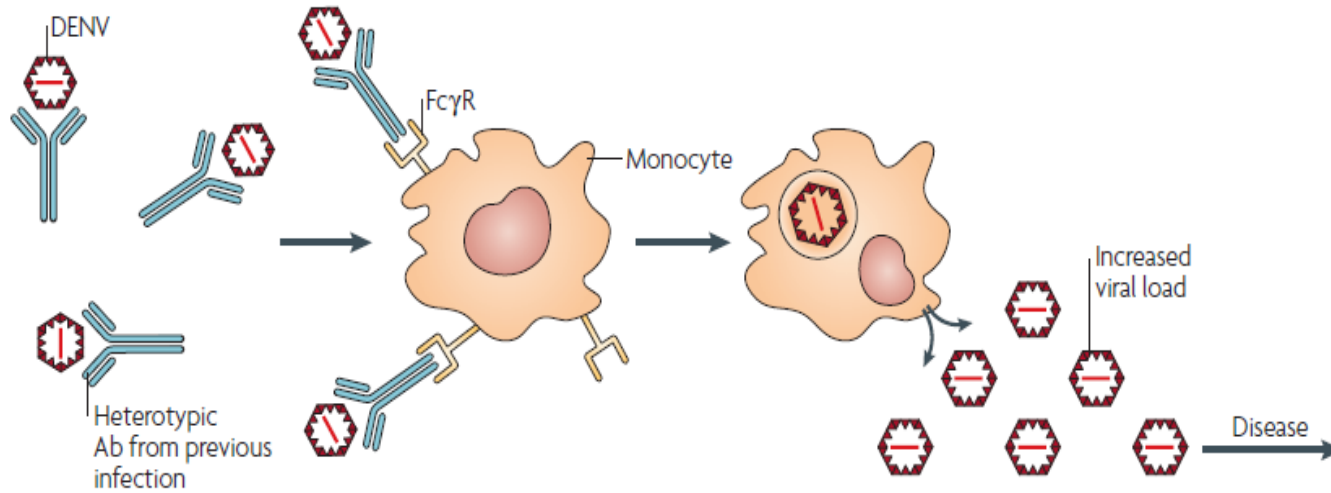
NT for DENV3

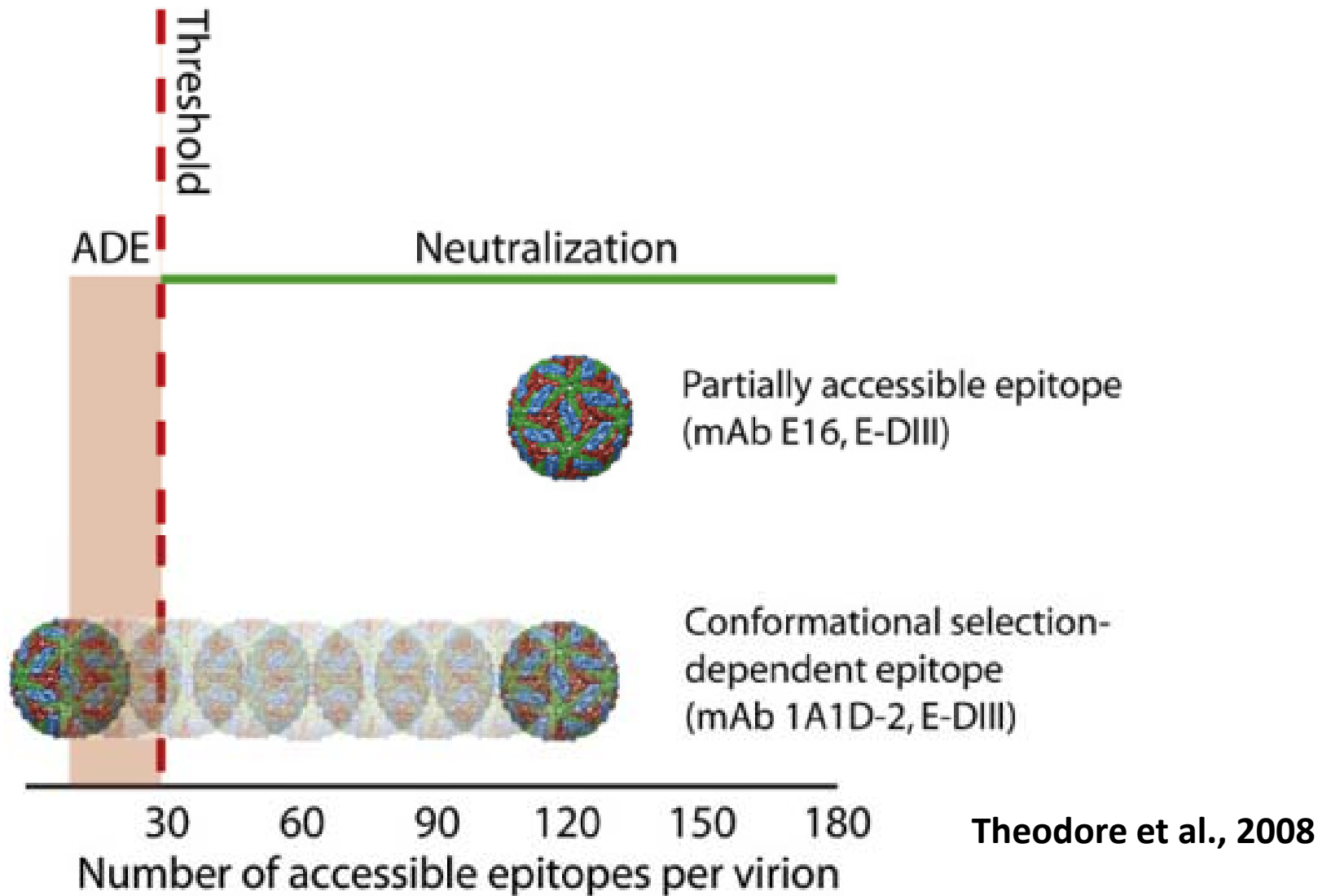


NT for DENV4

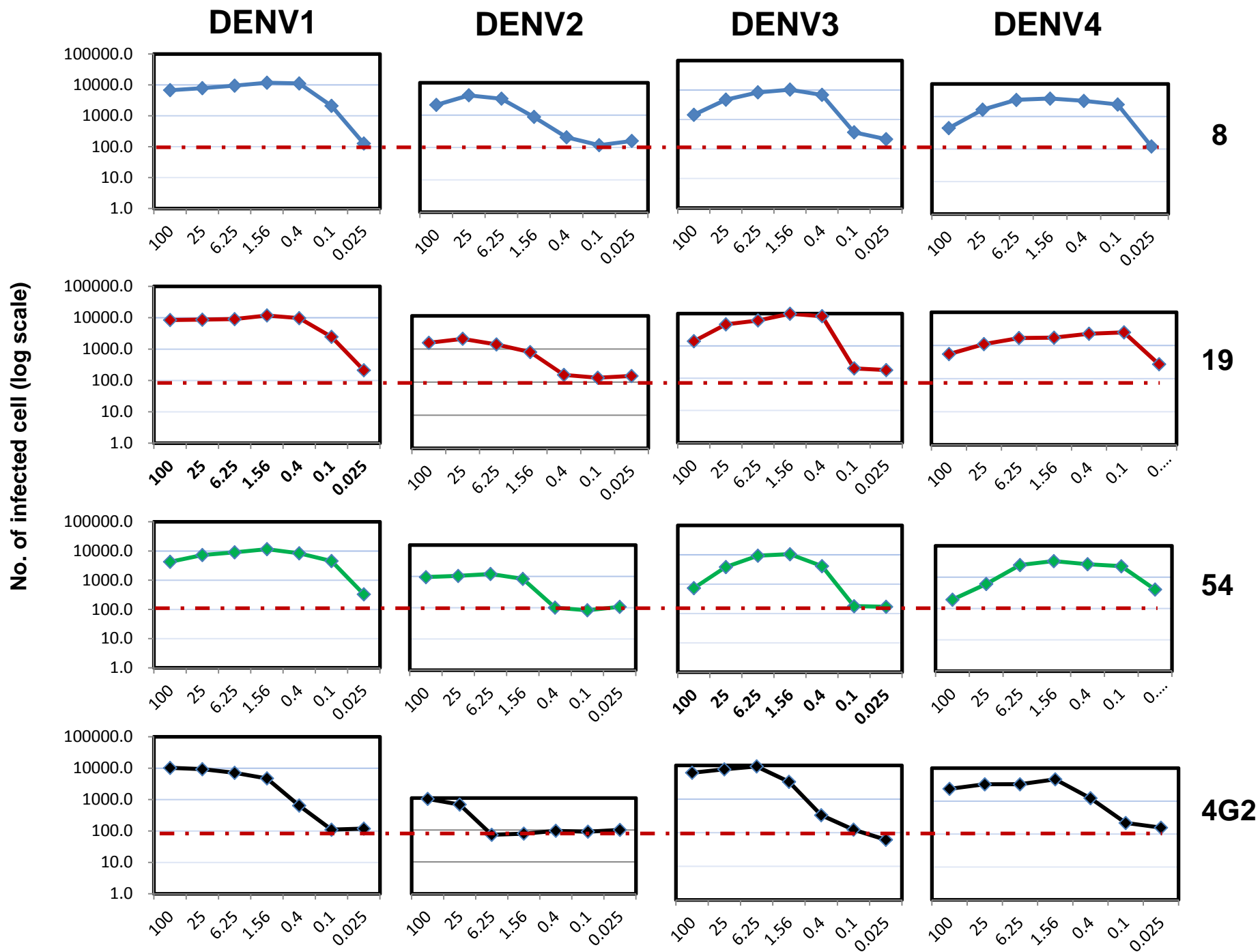


Antibody Dependent Enhancement

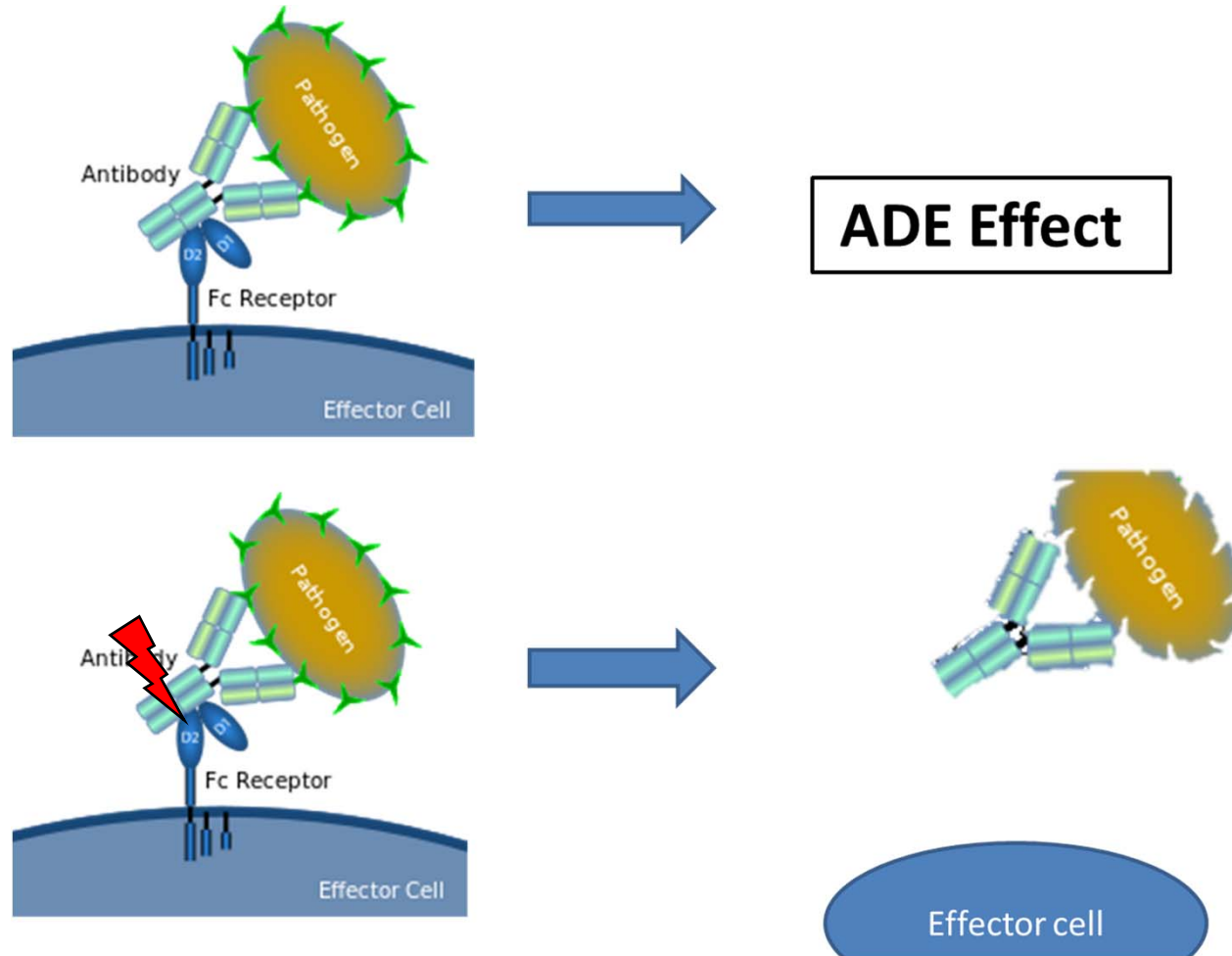




Several MAbs were reported to have ADE activity at sub-neutralizing concentration, which cause more severe form of DV (DHF and DSS) (Halstead, 2013; Schmidt, 2010; Lei et al., 2001)



ADE effect vs Fc Receptor



Balsitis et al., 2010
Williams et al., 2013

Fc engineered to reduce ADE

- Study in murine MAb, mutation at N297Q (Balsitis et al., 2010, and L234A/L235A (Williams et al., 2013) can reduce the binding of Fc and Fc gamma receptor, then reduce ADE
- One HuMAb (B3B9) were selected for rIgG production of wildtype/N297Q/LALA

Isolation of VH/VL from hybridoma cells

Hybridoma cell

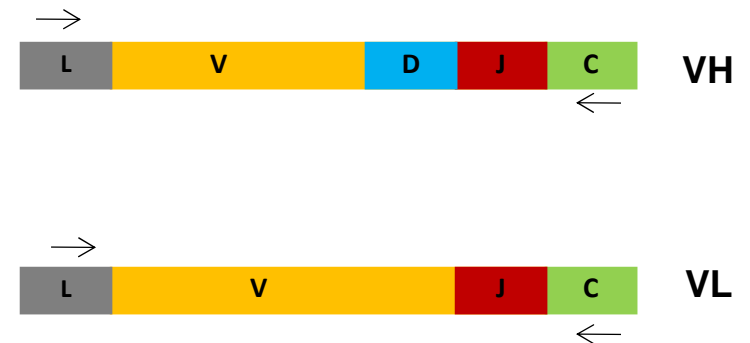
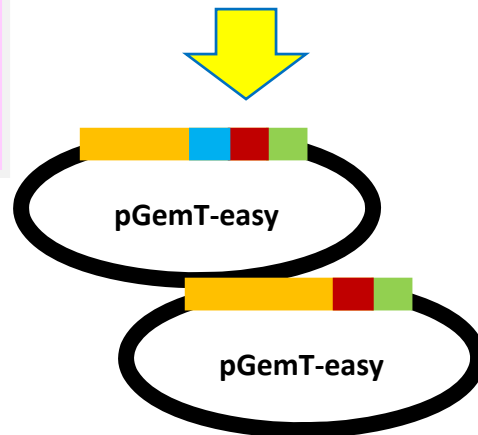


RNA isolation and cDNA synthesis

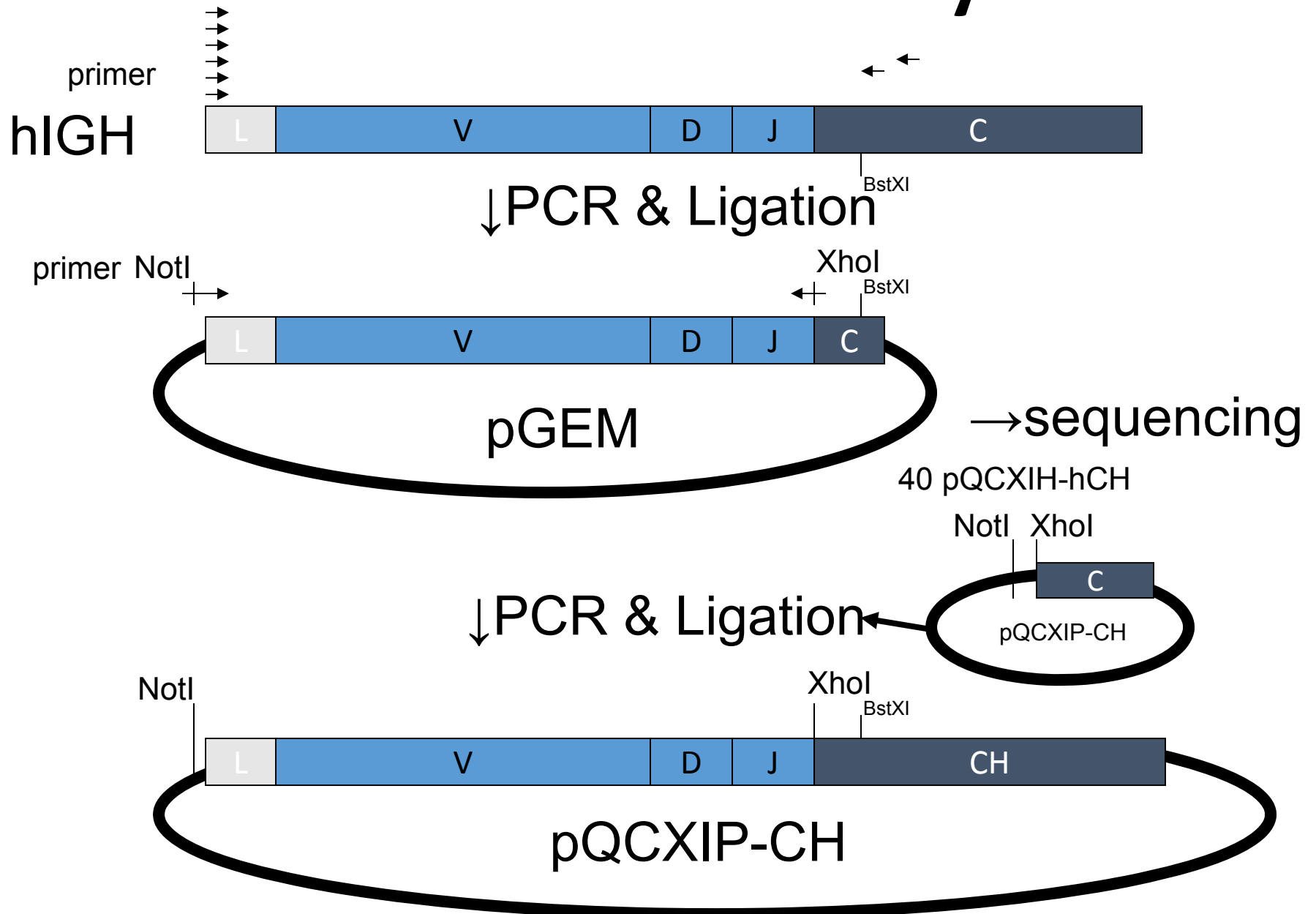


VH, VL amplification using 26 pairs of primer

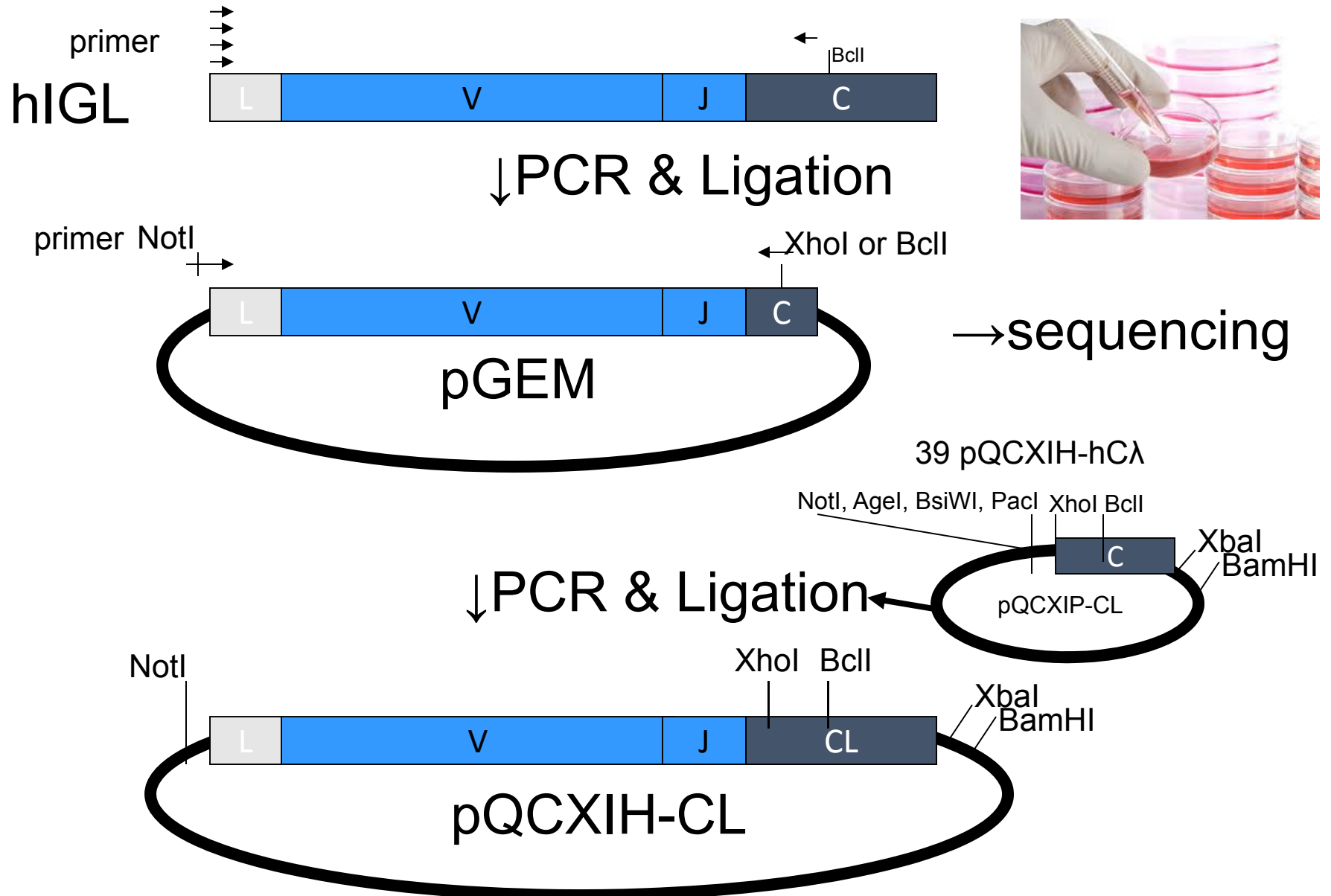
- 12 reactions for VH
- 10 reactions for V kappa
- 4 reactions for V lambda

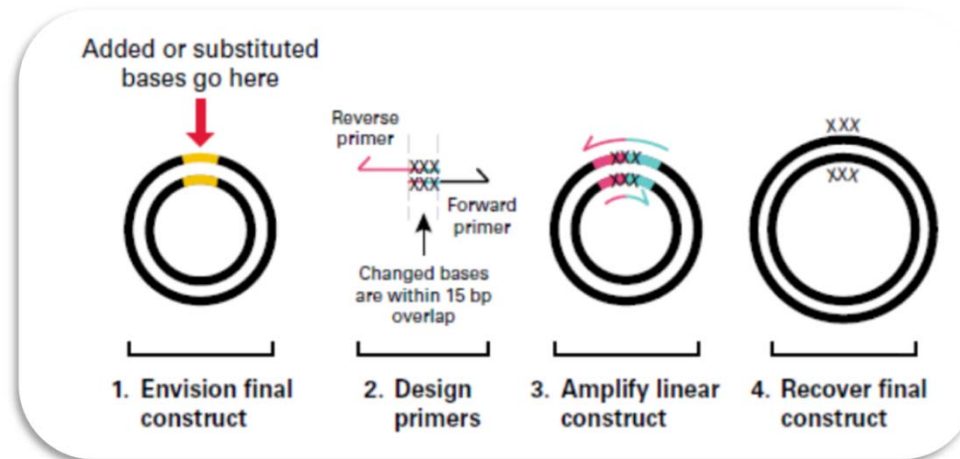


Heavy Chain



Lambda light chain





234 235

19WT: TGTGACAAACTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGG
 C D K T H T C P P C P A P E L L G

234 235

19LALA: TGTGACAAACTCACACATGCCACCGTGCCAGCACCTGAAAGCCGCCGGG
 C D K T H T C P P C P A P E A A G

297

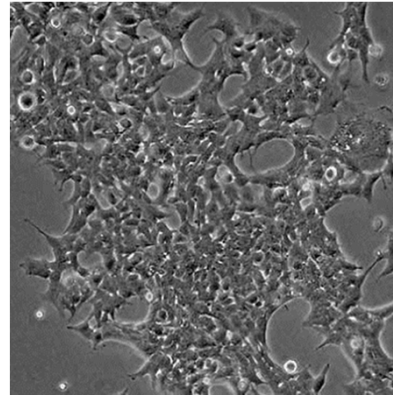
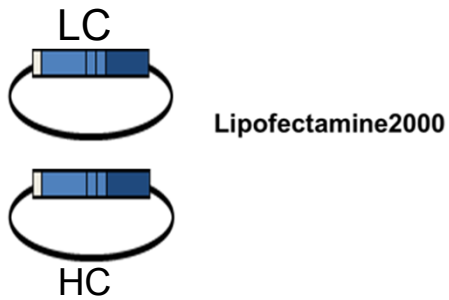
19WT: CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC
 R E E Q Y N S T Y R V V S V L T V

297

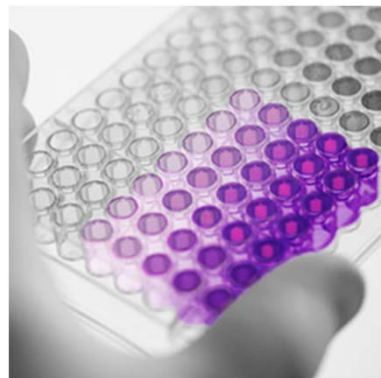
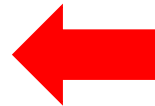
19N297Q: CGGGAGGAGCAGTACAGAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC
 R E E Q Y Q S T Y R V V S V L T V

Expression of modified IgG in mammalian cell

Co-transfection



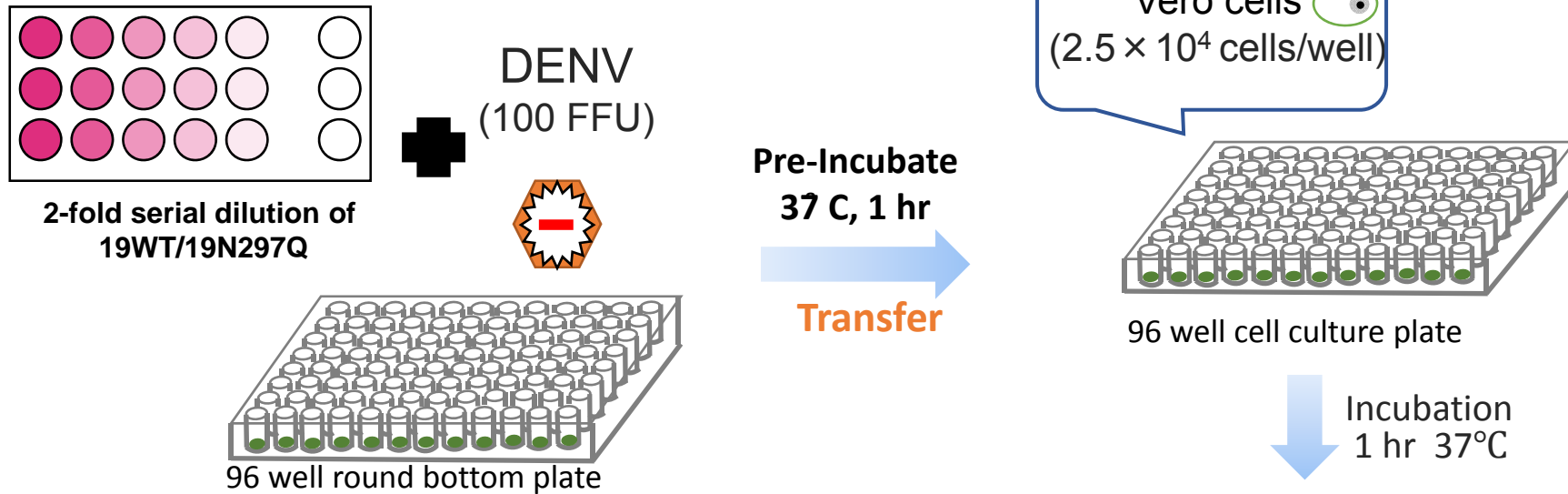
HEK293T



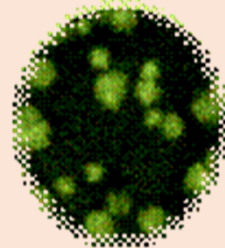
Measure concentration of antibody by BCA protein assay kit

Using Protein A affinity column

Neutralization assay

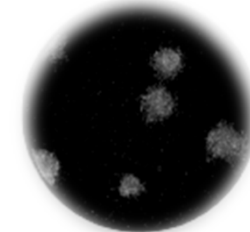


Neutralizing activity was expressed as **percentages of focus reduction** when compared to the virus control without Ab.



Add an overlay medium and further incubate for 2-3 day

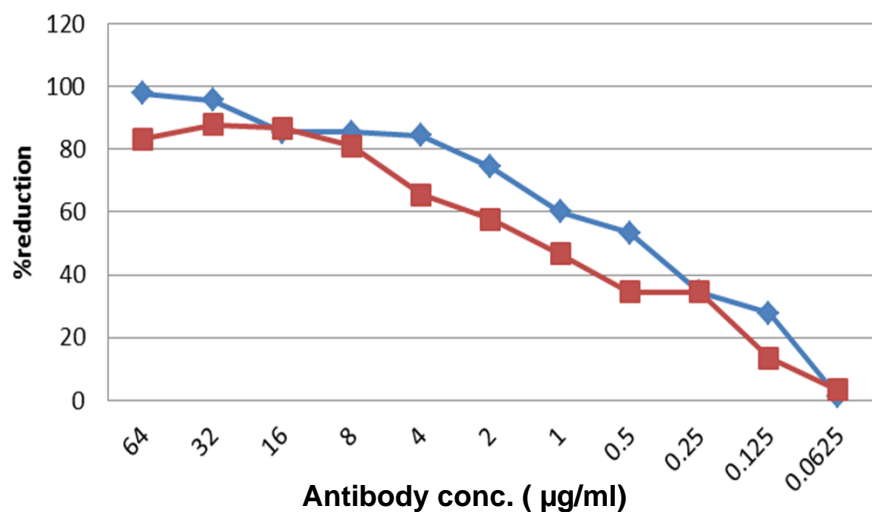
Immuno-stain by IFA



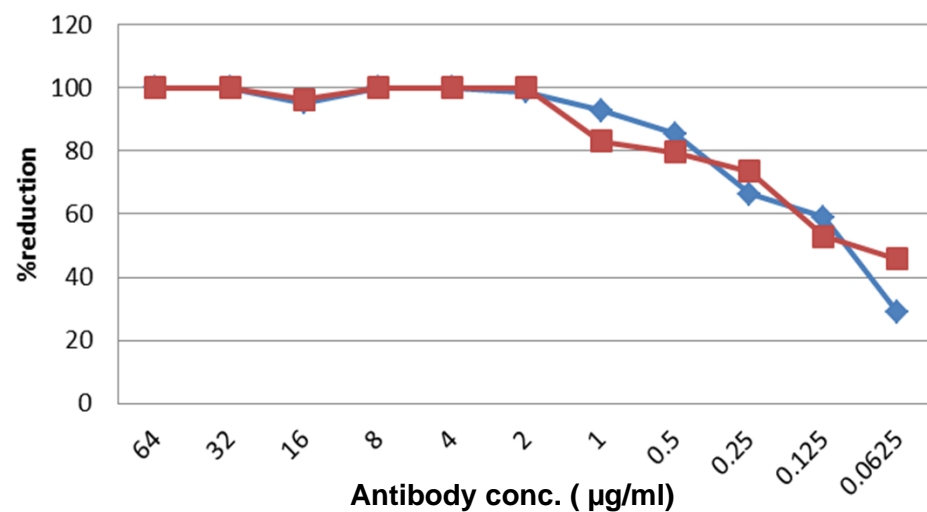
Count focus-forming unit (FFU) number

—◆— rIgG
 —■— Modified_Mab

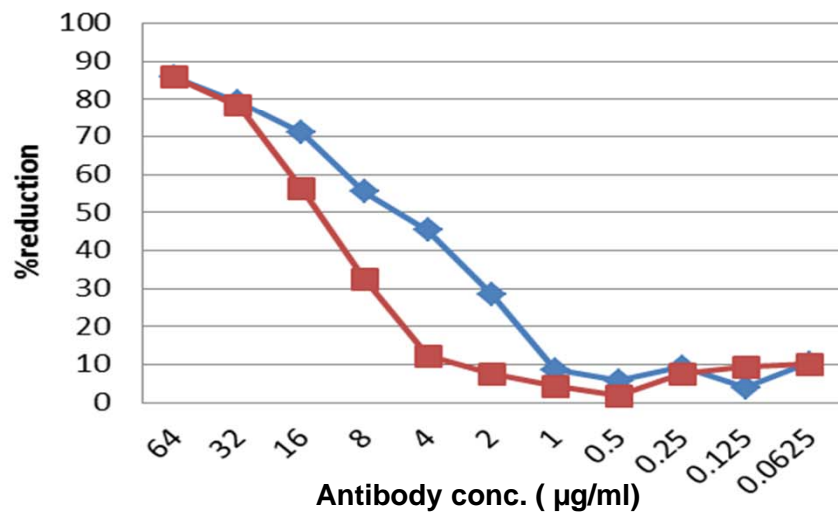
Neutralization test DV1



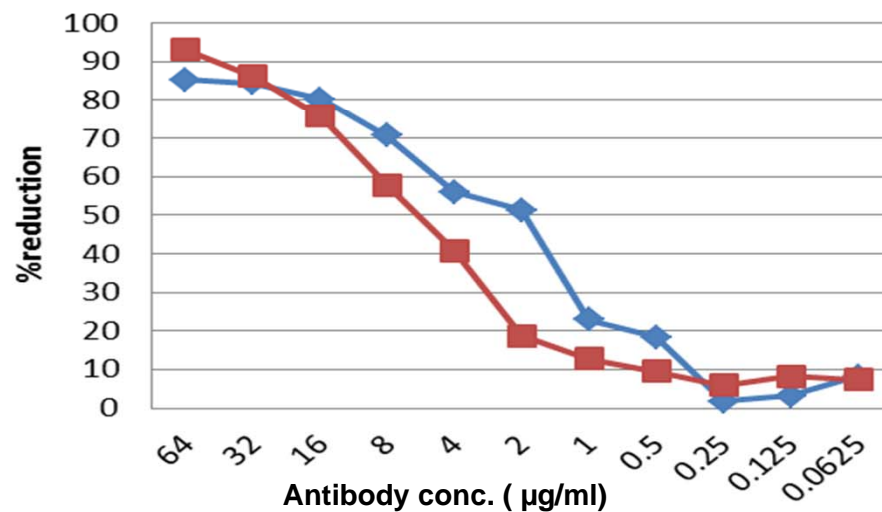
Neutralization test DV2



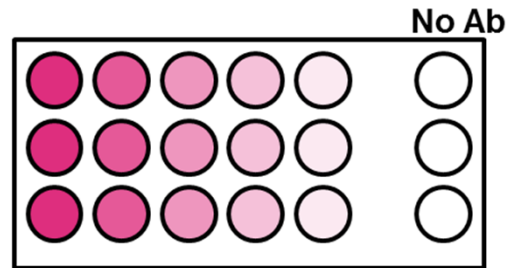
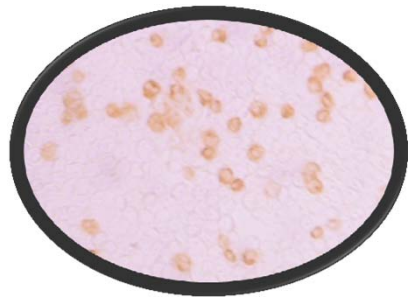
Neutralization test DV3



Neutralization test DV4



Antibody Dependent Enhancement assay by K562 (Containing FcγRIIa)



4- fold serial dilution of HuMAbs
in (+)s RPMI



DENV



Incubate for 2 hrs, at 37 °C



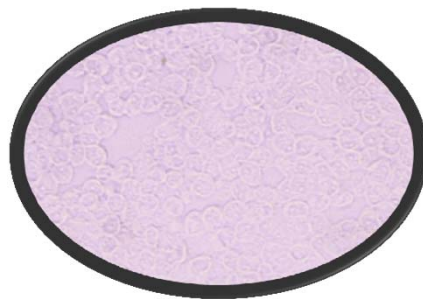
Add K562 cell at 1×10^5 cells /well



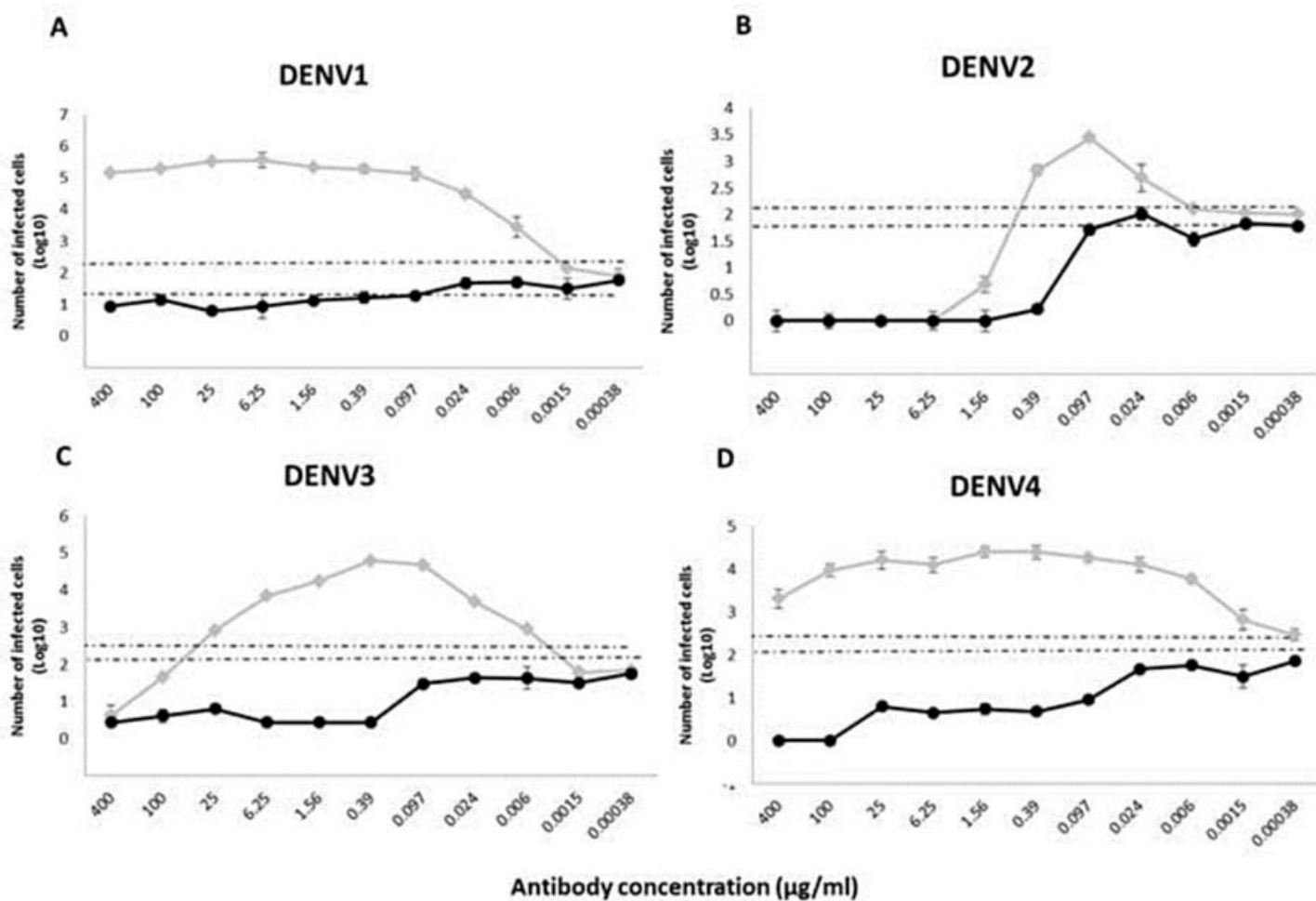
Incubate for 2 days



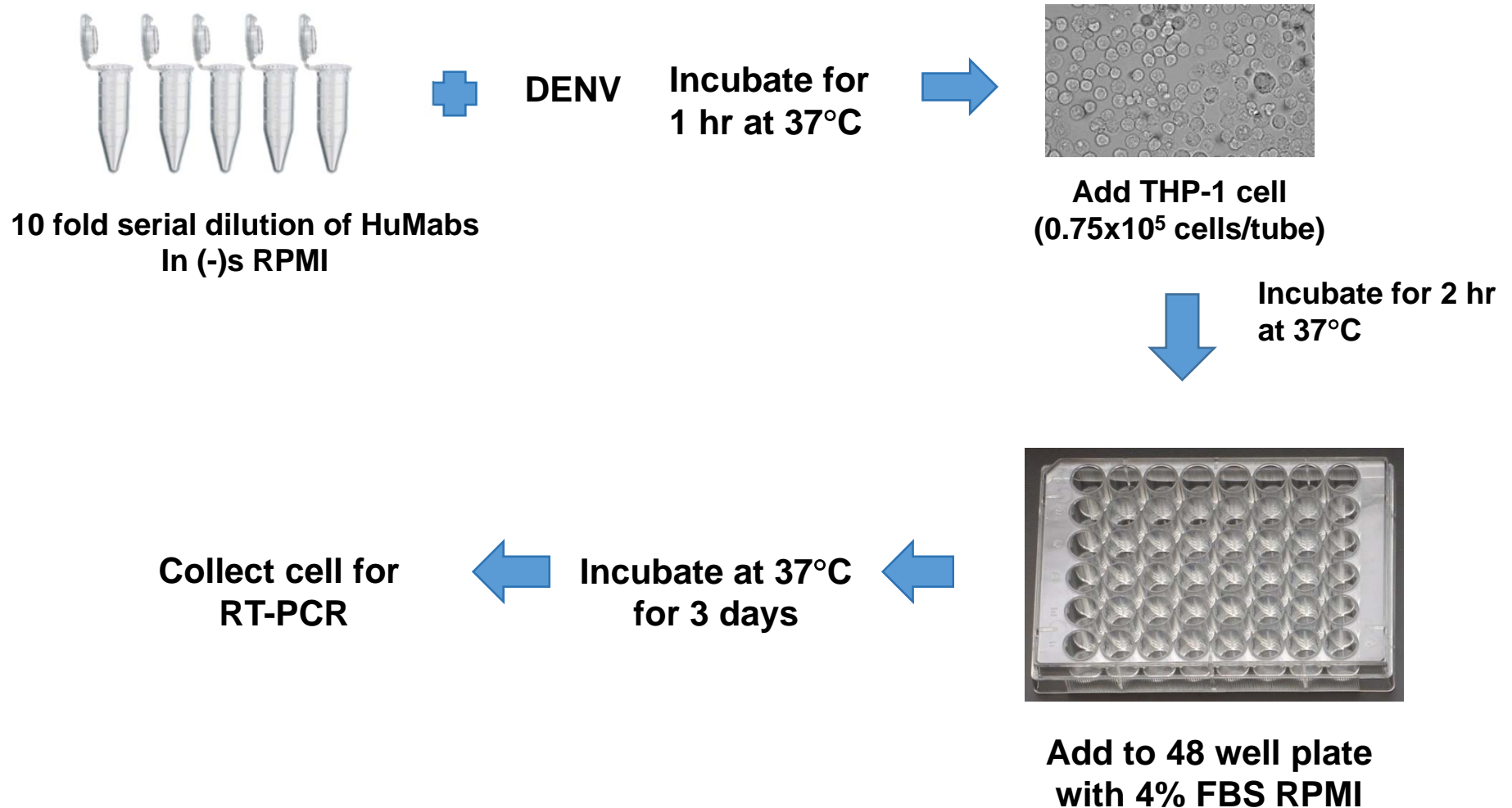
Immunostained



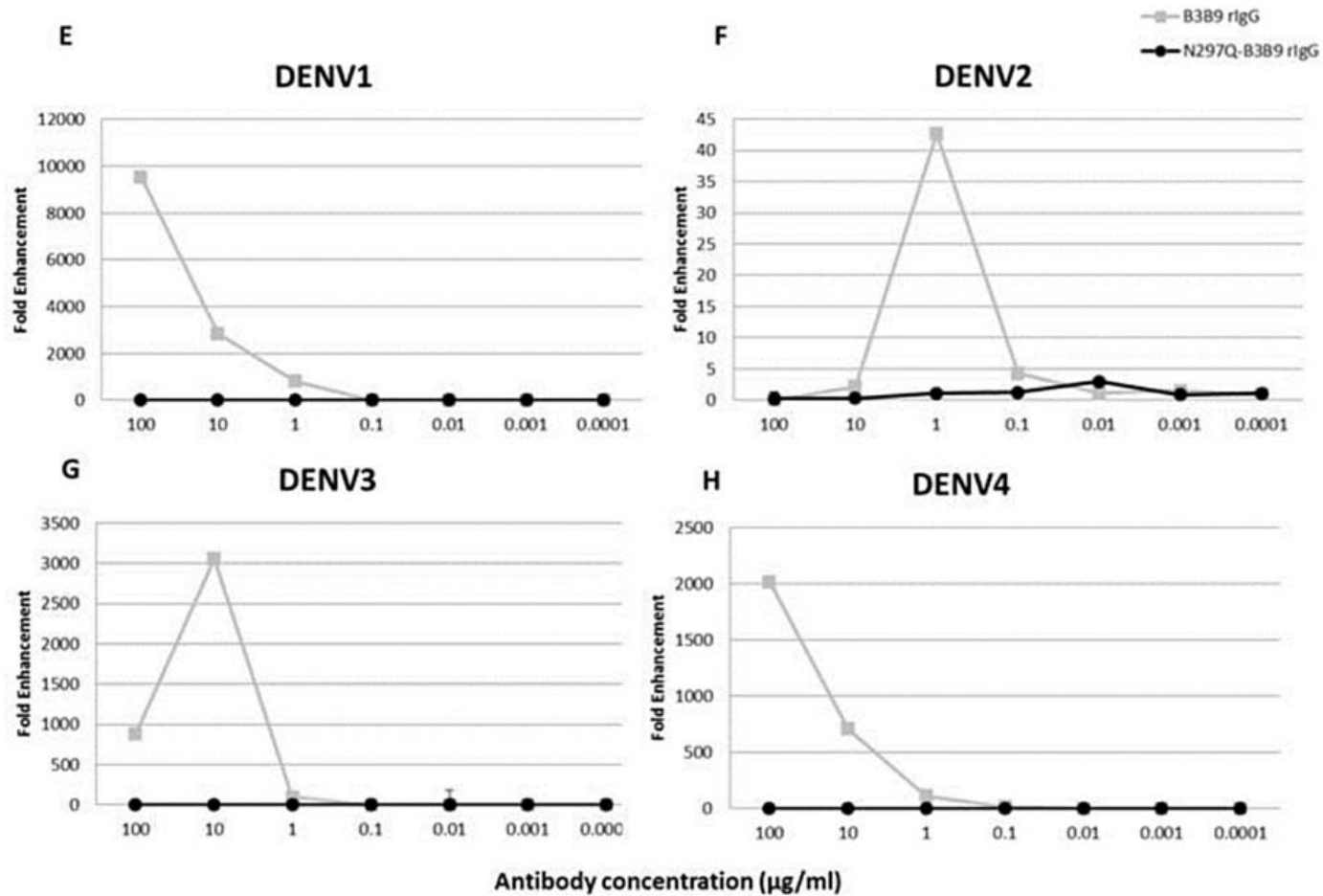
ADE study on K562 cell



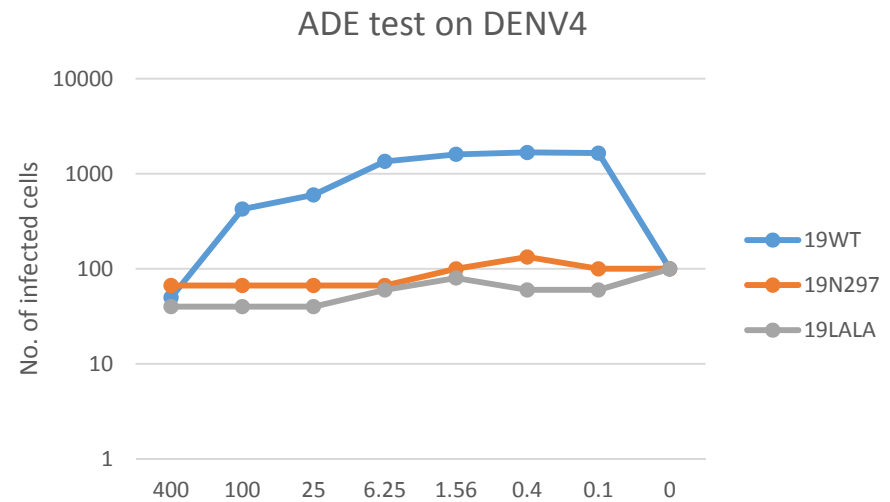
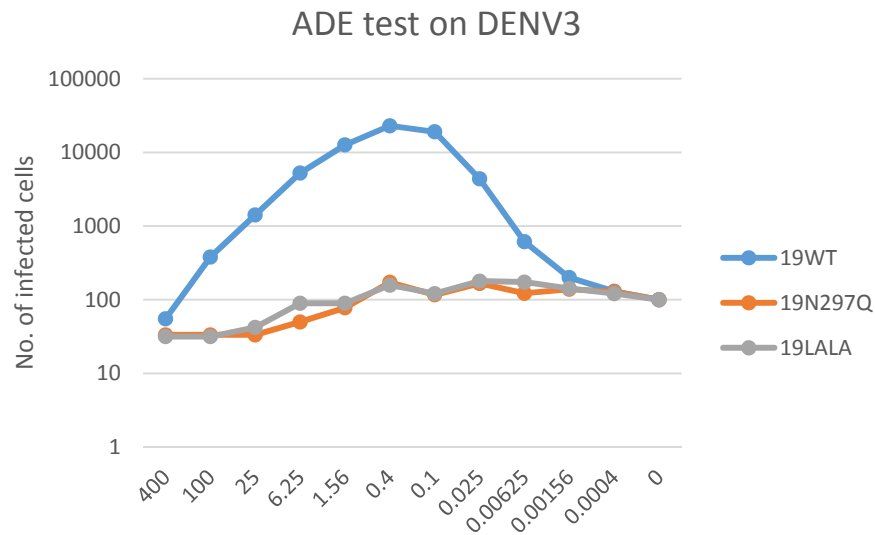
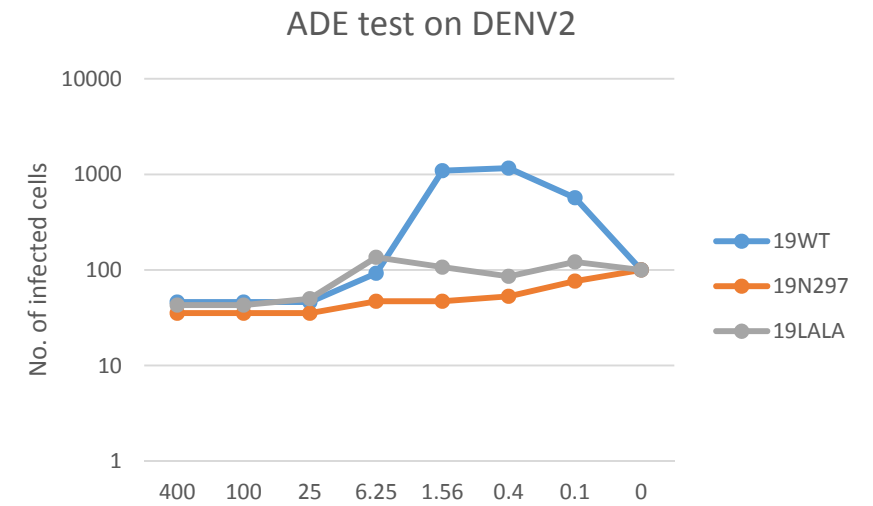
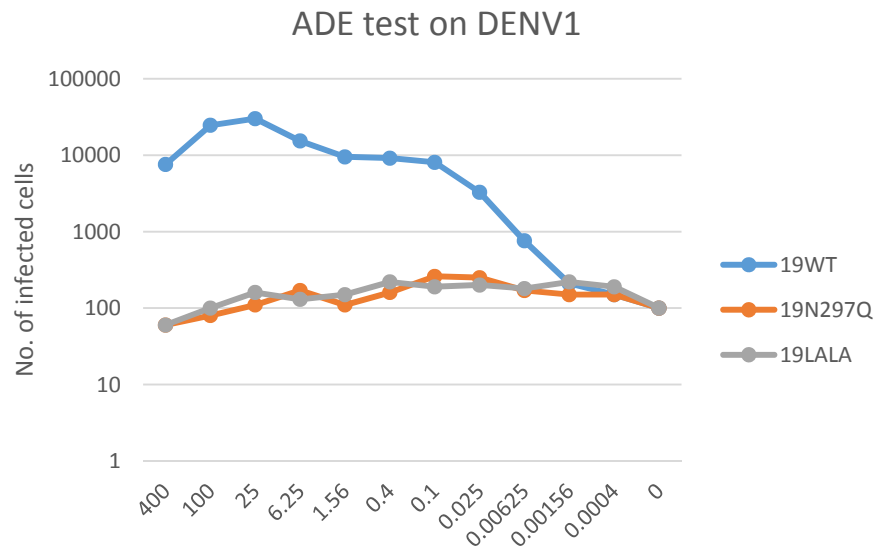
Antibody Dependent Enhancement assay by THP-1 cell (containing Fc γ RI/II)



ADE study on THP-1 cell



ADE study of 3 HuMAbs (WT, N297Q, LALA)



Generation and characterization of cross neutralizing human monoclonal antibody against 4 serotypes of dengue virus without enhancing activity

Subenya Injampa^{1,2}, Nataya Muenngern¹, Chonlatip Pipattanaboon¹,
Surachet Benjathummarak¹, Khwanchit Boonha¹, Hathairad Hananantachai²,
Waranya Wongwit², Pongrama Ramasoota^{1,2} and Pannamthip Pitaksajjakul^{1,2}

¹Center of Excellence for Antibody Reserach, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

²Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

ABSTRACT

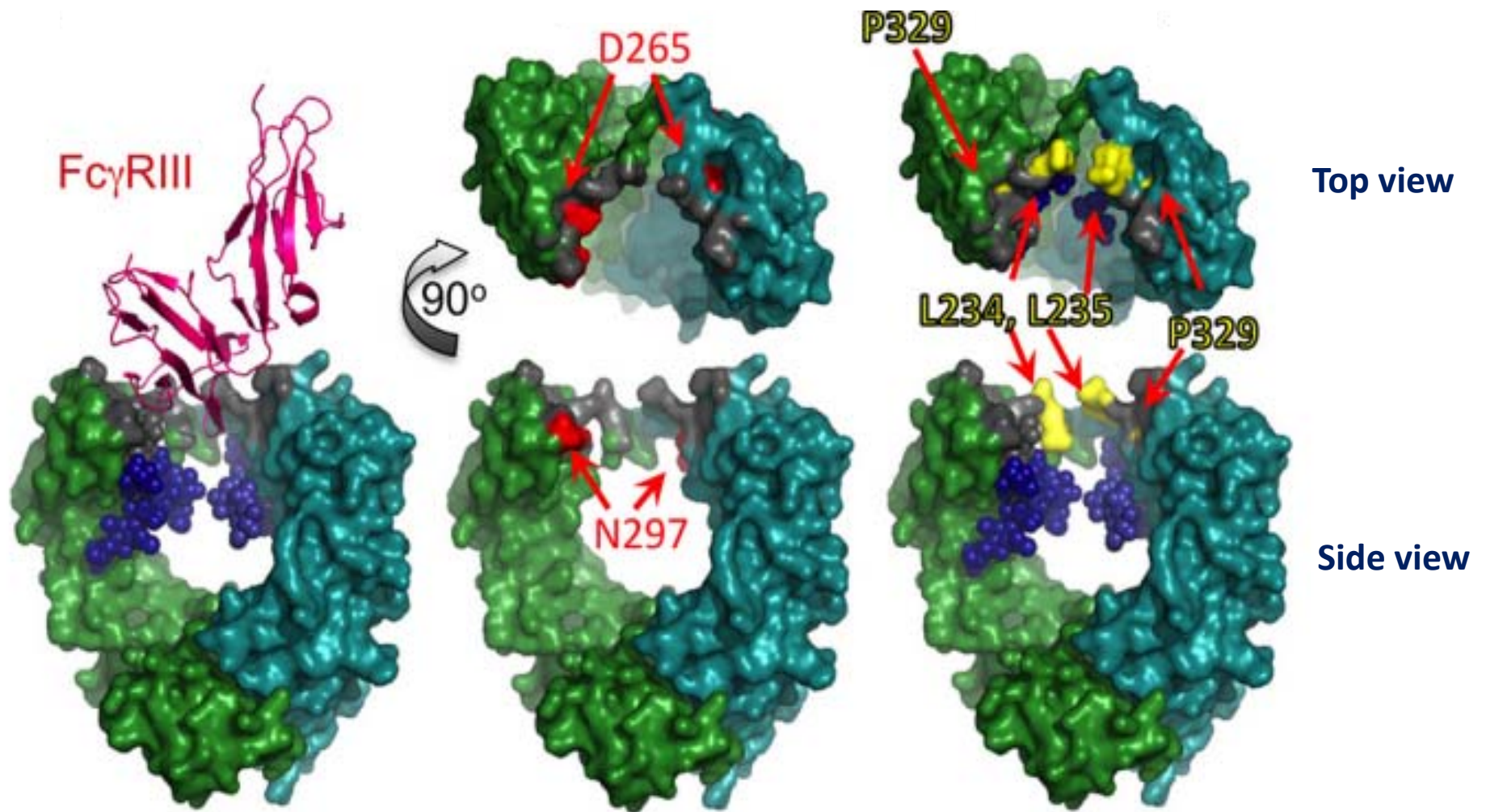
Background. Dengue disease is a leading cause of illness and death in the tropics and subtropics. Most severe cases occur among patients secondarily infected with a different dengue virus (DENV) serotype compared with that from the first infection, resulting in antibody-dependent enhancement activity (ADE). Our previous study generated the neutralizing human monoclonal antibody, D23-1B3B9 (B3B9), targeting the first domain II of E protein, which showed strong neutralizing activity (NT) against all four DENV serotypes. However, at sub-neutralizing concentrations, it showed ADE activity *in vitro*.

Methods. In this study, we constructed a new expression plasmid using the existing IgG heavy chain plasmid as a template for Fc modification at position N297Q by site-directed mutagenesis. The resulting plasmid was then co-transfected with a light chain plasmid to produce full recombinant IgG (rIgG) in mammalian cells (N297Q-B3B9). This rIgG was characterized for neutralizing and enhancing activity by using different FcγR bearing cells. To produce sufficient quantities of B3B9 rIgG for further characterization, CHO-K1 cells stably secreting N297Q-B3B9 rIgG were then established.

Results. The generated N297Q-B3B9 rIgG which targets the conserved N-terminal fusion loop of DENV envelope protein showed the same cross-neutralizing activity to all

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Corresponding author
Pannamthip Pitaksajjakul,



N297Q: Aglycosylation substitution caused allosteric effect
LALA: form part of FcγR binding site

Conclusion and Further study

- 19N297Q and 19LALA showed comparable result in reducing ADE studied in K562 cells
- Aglycosylation of 19N297Q may effect to antibody expression and assembly
- Expression and stability of both modified rIgG needed to be identified

Conclusion and Further study

- Mice lacking all Fc receptor required higher dose of NuAbs to maintain equivalent level of protection in case of WNV (Oilphant et al., 2006)
- Fc mediated Effector function (CDC, ADCC, ADCP) should be evaluated *in vitro* and *in vivo*
- Different mutation showed different affinity to Fc receptor, and effector functions (Lo et al., 2017)
- **Substitution at other positions to increase effector functions (D270, K322, P329, P331) (Saxena and Wu, 2016) can be studied**

Acknowledgement

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- MOCID (Mahidol-Osaka Center for Infectious Diseases)
- Prof. Eiji Konishi/ Asst. Dr. Atsushi Yamanaka
- International Center for Biotechnology, Osaka University



**Thank you
for your attention**