

เสวนาพิเศษเนื่องในโอกาส ..

วันคล้ายวันสถาปนา คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล 29 มิถุนายน และวันเทคนิคการแพทย์ไทย



มหาวิทยาลัยมหิดล
คณะเทคนิคการแพทย์

“ COVID-19 vaccine : การตรวจภูมิตอบสนอง จำเป็นแค่ไหน ? ”

- ภูมิตอบสนอง vs ภูมิคุ้มกัน หลังฉีดวัคซีน
- การตรวจ Binding antibody และ Neutralizing antibody

วันอังคารที่ 29 มิถุนายน 2564
เวลา 10.00 - 12.00 น.

ถ่ายทอดสดผ่าน LIVE

Faculty of Medical Technology, Mahidol University (MUMT)

<https://www.facebook.com/MedTechMU>

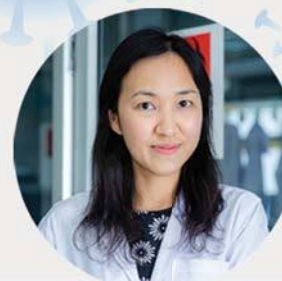
วิทยากร



ศ.เกียรติคุณ ดร.ไพฑิณี พุฒิตนนะ
ที่ปรึกษาศูนย์วิจัยพัฒนานวัตกรรม
คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล
นายกสมาคมไวรัสวิทยา (ประเทศไทย)



ผศ. ดร.ชนิยา ลีปิยะสกุลชัย
ภาควิชาจุลชีววิทยาคลินิกและเทคโนโลยีประยุกต์
คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล



ผศ. ดร.กทียรัตน์ เลิศสำราญ
ศูนย์วิจัยพัฒนานวัตกรรม
คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล

สปนลงทะเบียนเข้าร่วมงาน

อยู่ระหว่างการดำเนินการขอ CMTE



ฟรี

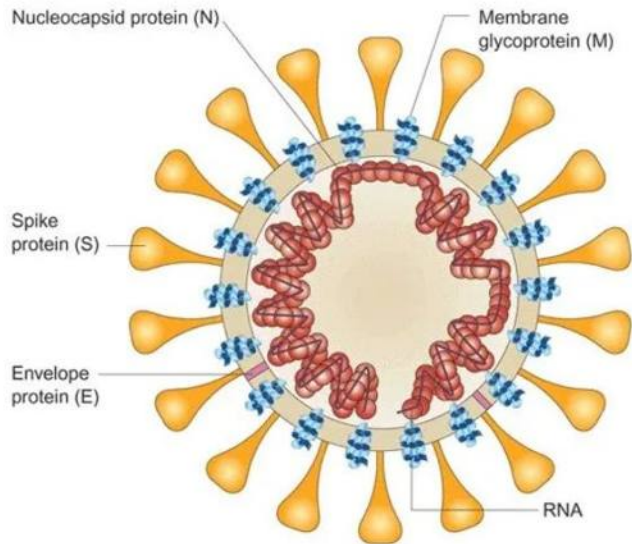
COVID-19
NOVEL CORONAVIRUS
DISEASE 2019 VAC



Current COVID 19- Vaccines & Immune responses



SAR CoV-2 structure

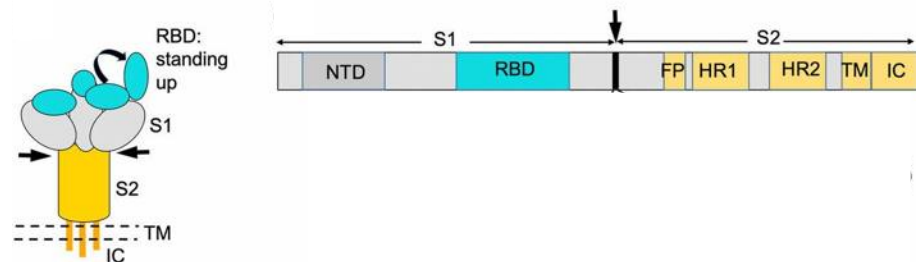


ss RNA genome at length of 29, 903 kb comprises 14 ORFs and encodes 16 nonstructural proteins (NSP1-16), 4 structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins, and 9 accessory proteins.



ORF1a	4405 aa
ORF1b	2595 aa
S	1282 aa
ORF3a	275 aa
E	75 aa
M	222 aa
ORF6	61 aa
ORF7a	121 aa
ORF8	121 aa
ORF10	419 aa

Wuhan-Hu-1 (GenBank MN908947)





สถานะวัคซีนโควิด-19 ของประเทศไทย

บริษัทยา	ชื่อวัคซีนที่ขออนุมัติ	นำเข้าโดย	วันที่อนุมัติ
	วัคซีนCOVID-19 VACCINE ARAZENECA	บริษัท แอสตราเซนเนกา (ประเทศไทย) จำกัด ผลิตโดย บริษัท สยามไบโอไซเอนซ์ จำกัด	20 ม.ค. 2564
	วัคซีนโคโรนาแวก / CoronaVac	องค์การเภสัชกรรม (อก.)	22 ก.พ. 2564
	วัคซีนโควิด-19 วัคซีนแจนเซน / COVID-19 Vaccine Janssen	บริษัท แจนเซน-ซีแอล จำกัด	25 มี.ค. 2564
	วัคซีนCOVID-19 VACCINE MODERNA	บริษัท ซิลลิคฟาร์มา จำกัด	13 พ.ค. 2564
	วัคซีนCOVID-19 Vaccine (Vero Cell) , Inactivated COVILO (BIBP)	บริษัท ไบโอจีเน็ด จำกัด	28 พ.ค. 2564
	วัคซีนโคเมอร์เนดี (COMIRNATY VACCINE)	บริษัท ไฟเซอร์ ประเทศไทย จำกัด	NEW 24 มิ.ย. 2564

อย. อนุมัติ วัคซีน โควิด-19 แล้ว (แบบใช้เข็ม ภาวะฉุกเฉิน)

วัคซีนที่อยู่ระหว่างดำเนินการ (เอกสารสำคัญยังไม่ครบ)

	วัคซีนSputnik V	บริษัท สกินเจน ไบโอบีท จำกัด	-
	วัคซีนCovaxin	บริษัท ไบโอบีเน็ด จำกัด	-

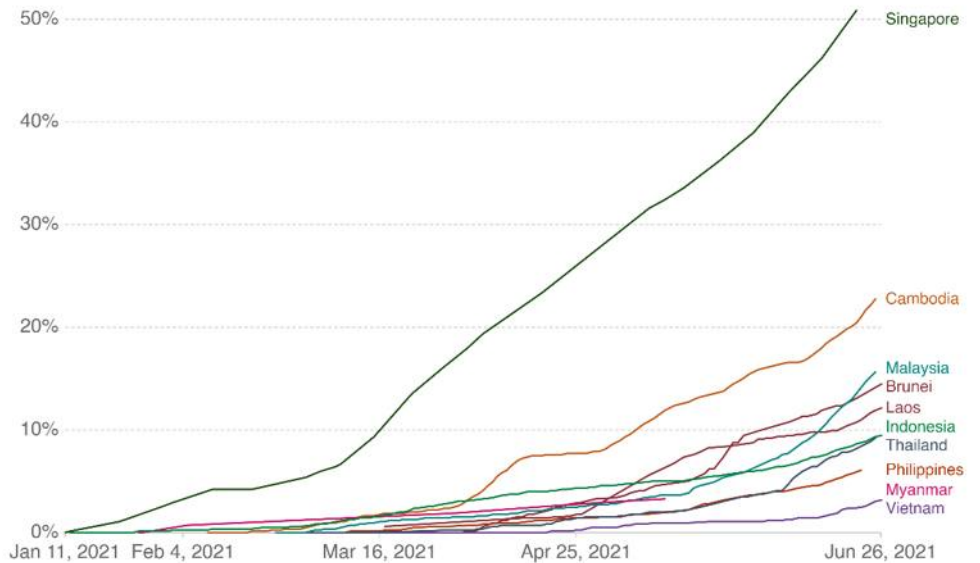
ผลิตโดย กองพัฒนาวิทยาศาสตร์และเทคโนโลยี
ข้อมูล ณ วันที่ 24 มิถุนายน 2564

/FDATHAI



Share of people who received at least one dose of COVID-19 vaccine

Share of the total population that received at least one vaccine dose. This may not equal the share that are fully vaccinated if the vaccine requires two doses.



Source: Official data collated by Our World in Data

CC BY

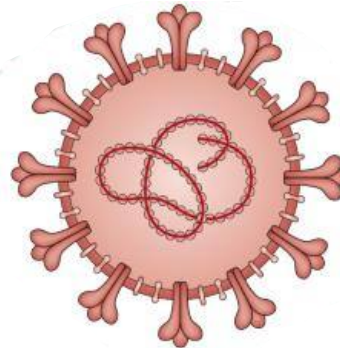
The different platforms of COVID-19 vaccines

Classical Platform

Whole- inactivated virus



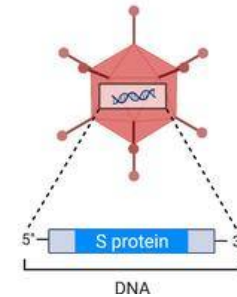
Sinopharm/Sinovac/
Covaxin



Next Generation Platform

Viral Vector

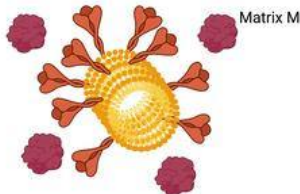
AstraZeneca



AstraZeneca/Johnson&Johnson/
SputnikV

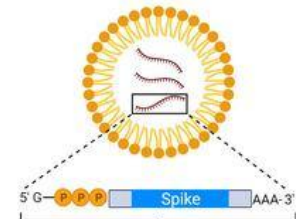
Protein subunit/ Virus like particle

Novavax



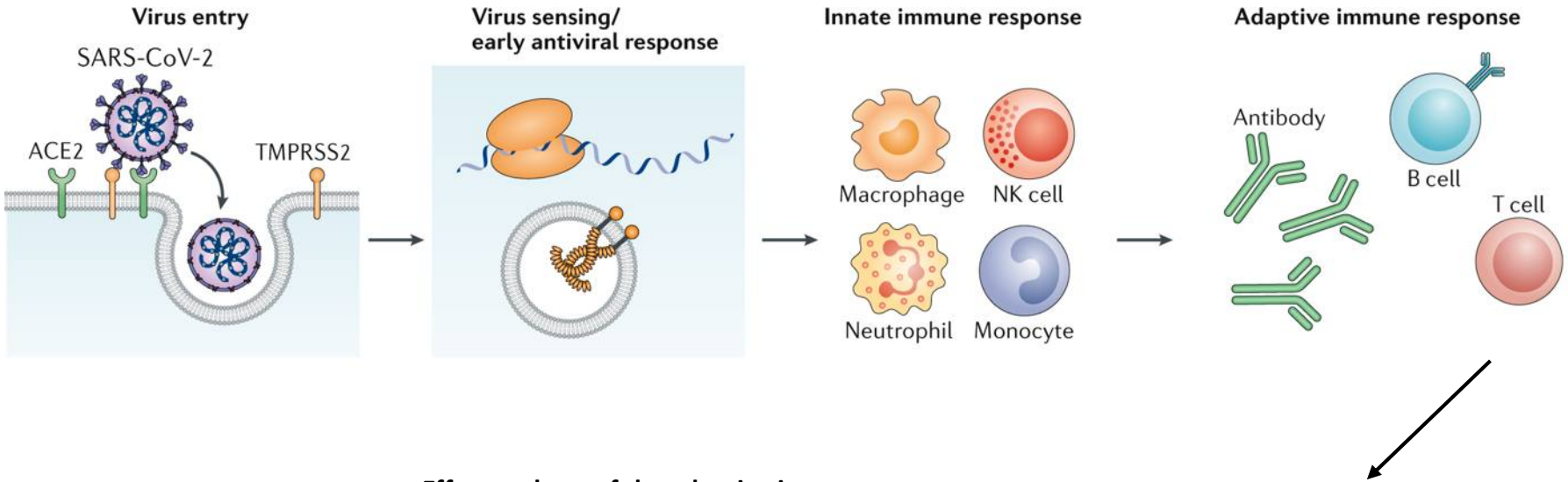
mRNA

BioNTech/Pfizer

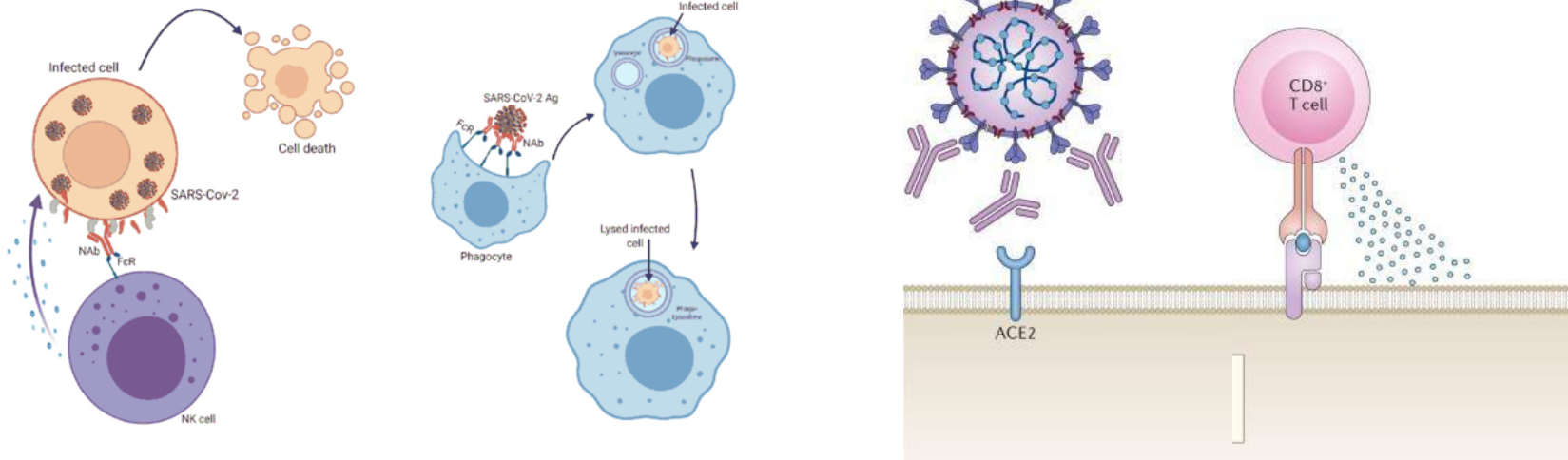


Moderna/Pfizer-BioNtech

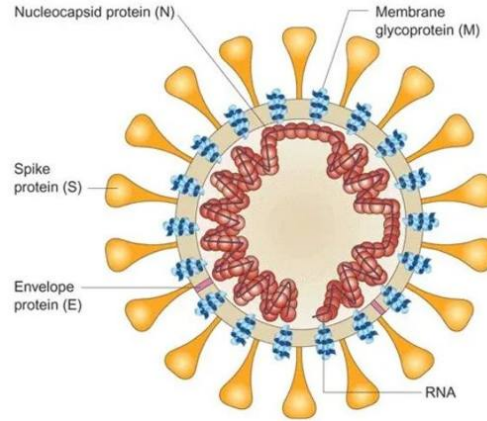
Basic immune responses to SAR CoV-2 infection



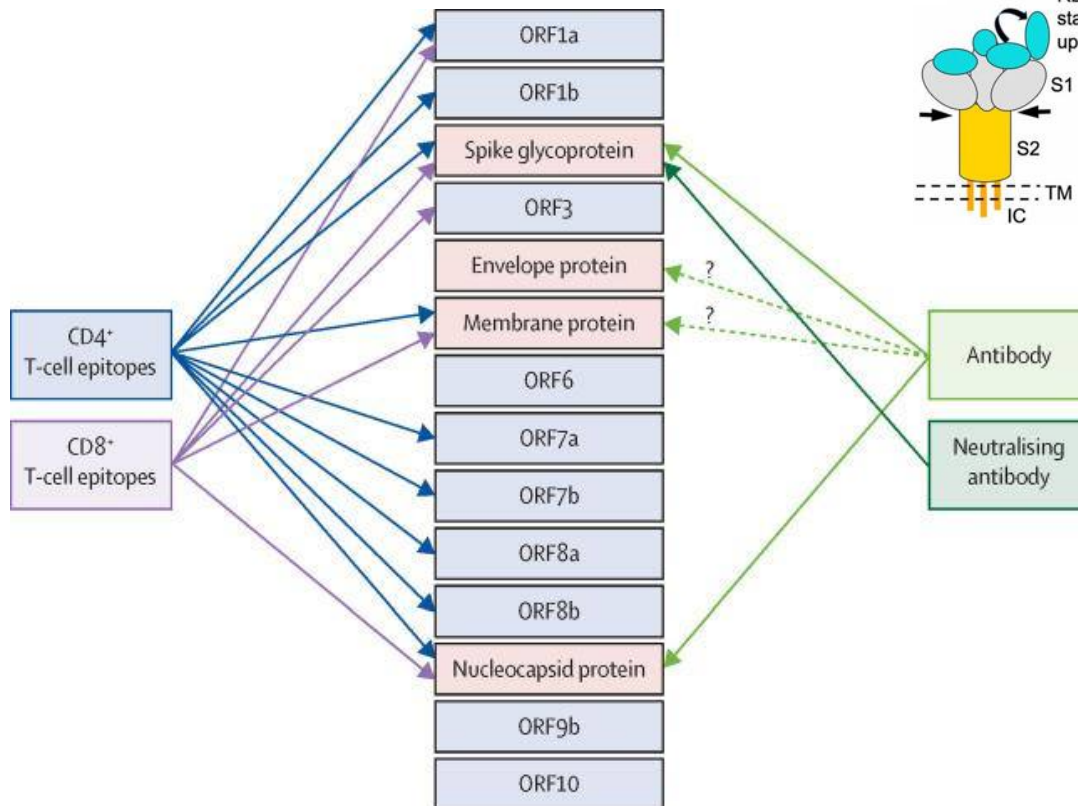
Effector phase of the adaptive immune response



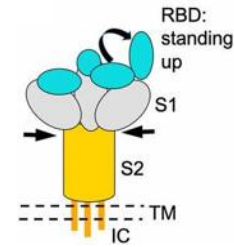
Basic immune responses to SAR CoV-2 infection



Specific T cells



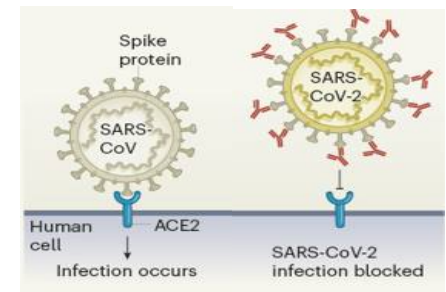
Neutralizing antibody or binding antibody?



Binding antibody

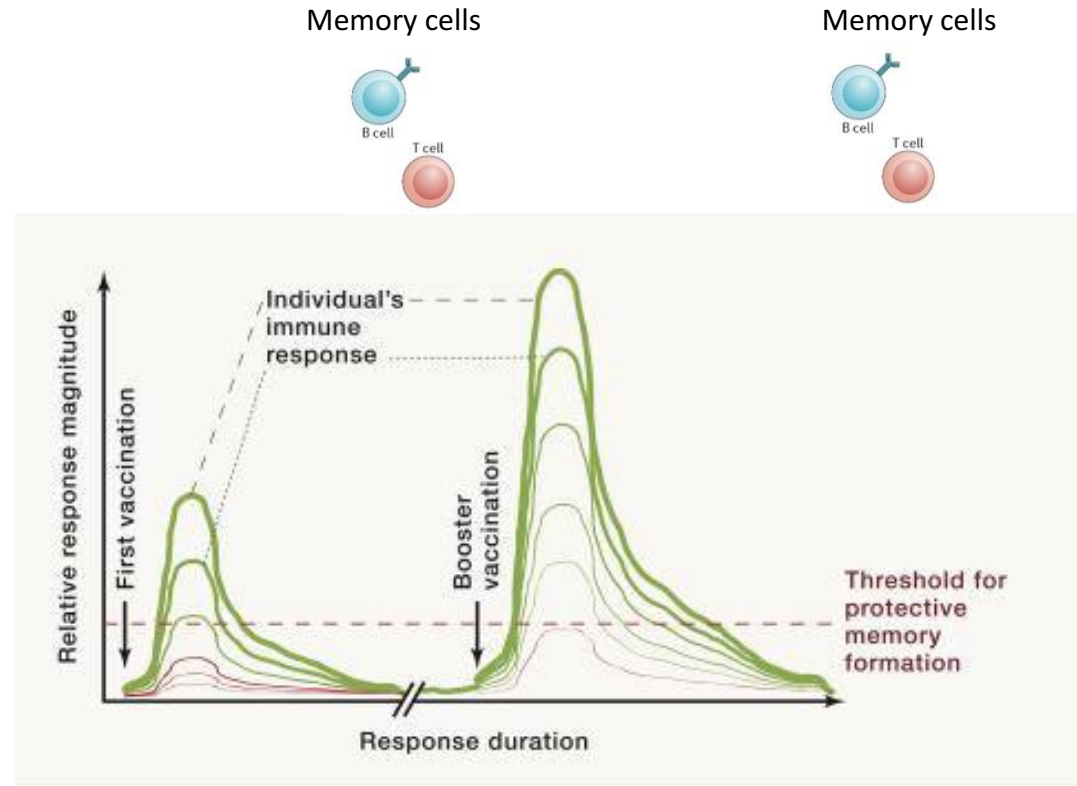
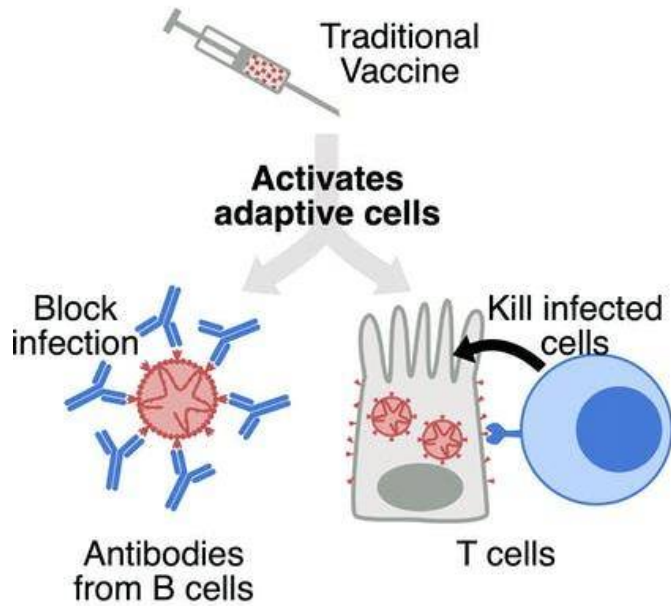
- Anti RBD
- Anti Spike protein
- Anti Nucleocapsid protein

Neutralizing antibody



An immune response is induced by vaccine

Immune Memory

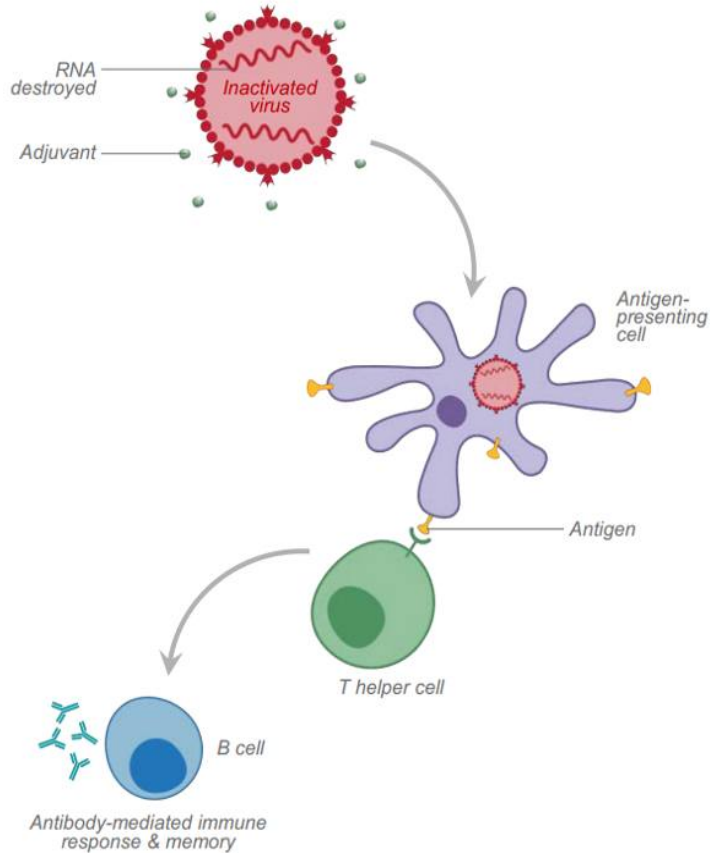


DOI: <https://doi.org/10.1016/j.immuni.2021.01.014>

An immune response is induced by vaccine

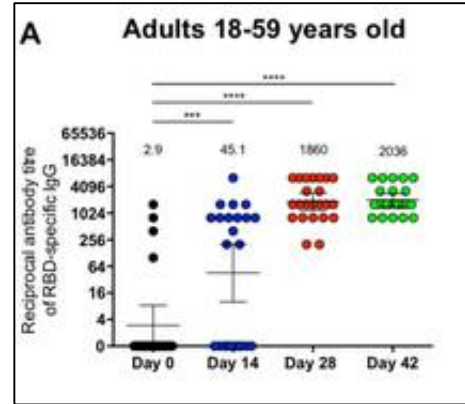
Inactivated virus vaccines

Sinopharm / Sinovac/Covaxin

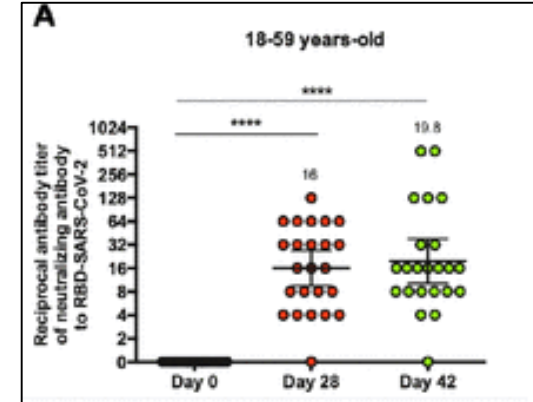


Antibody response after immunization with CoronaVac

Binding Ab

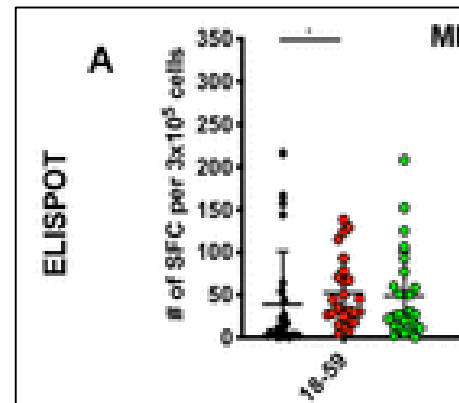


Neutralizing Ab

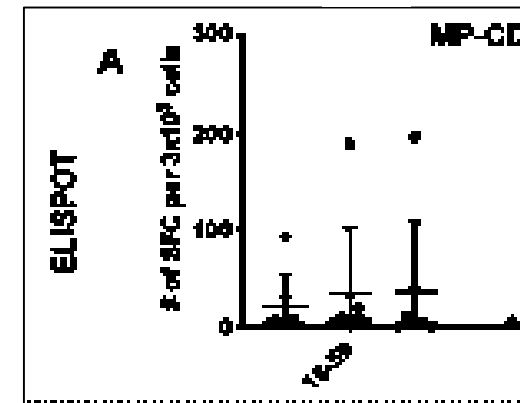


T cell response after immunization with CoronaVac

CD4



CD8



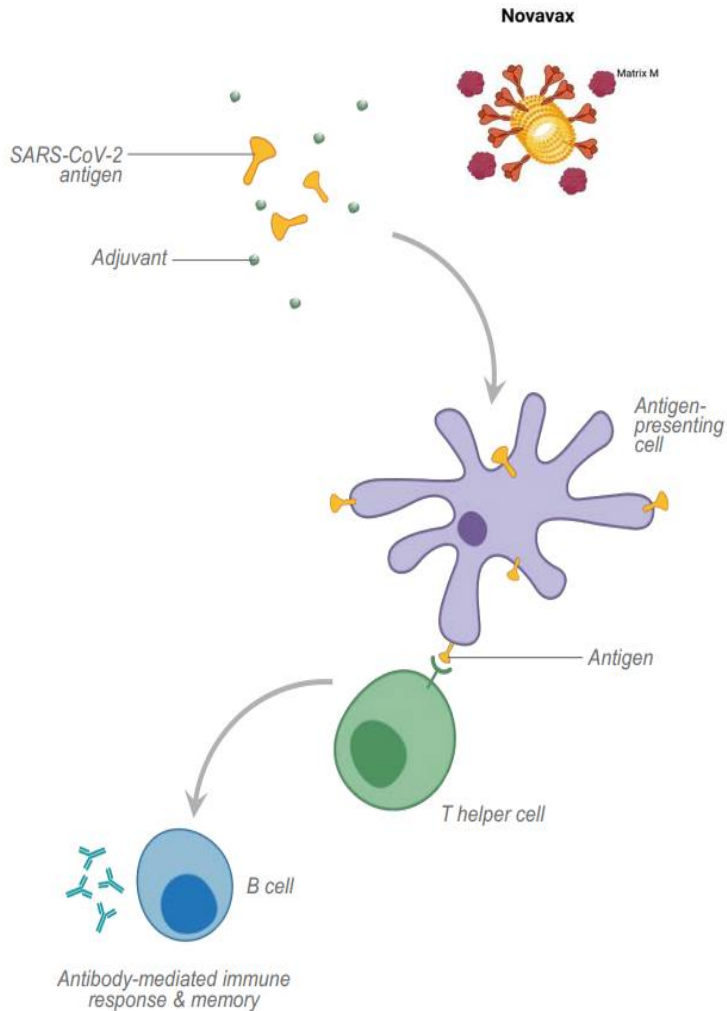
Interim report: Safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 in healthy Chilean adults in a phase 3 clinical trial

An immune response is induced by vaccine

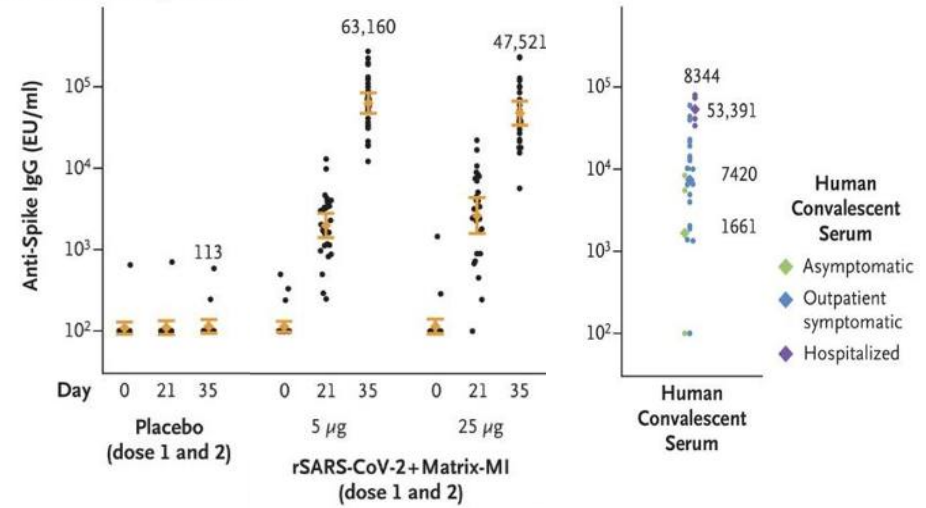


Viral subunit vaccines/Virus like particle

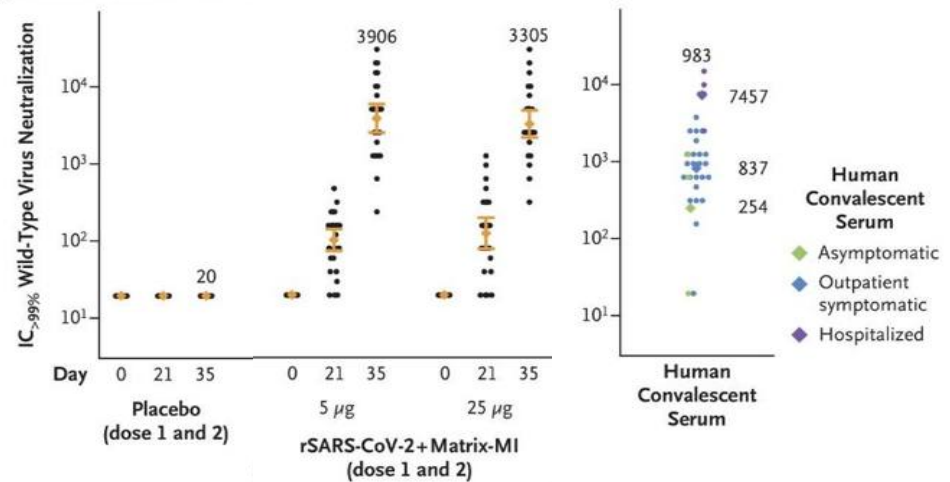
Novavax induced Spike IgG and Neutralizing Antibody Responses



A SARS-CoV-2 Anti-Spike IgG ELISA



B Wild-Type SARS-CoV-2 Microneutralization

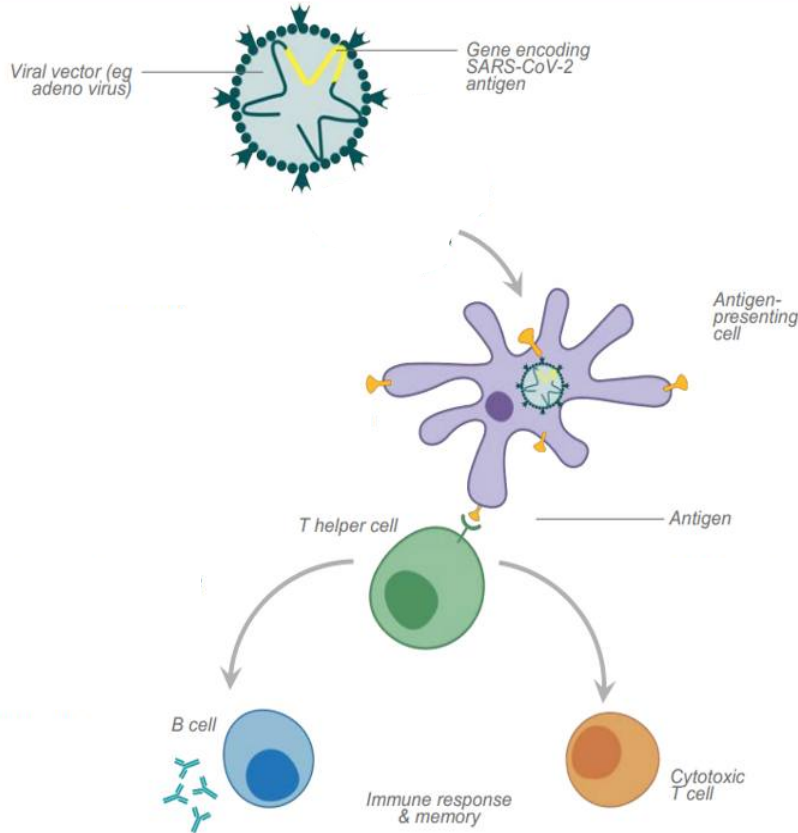


An immune response is induced by vaccine

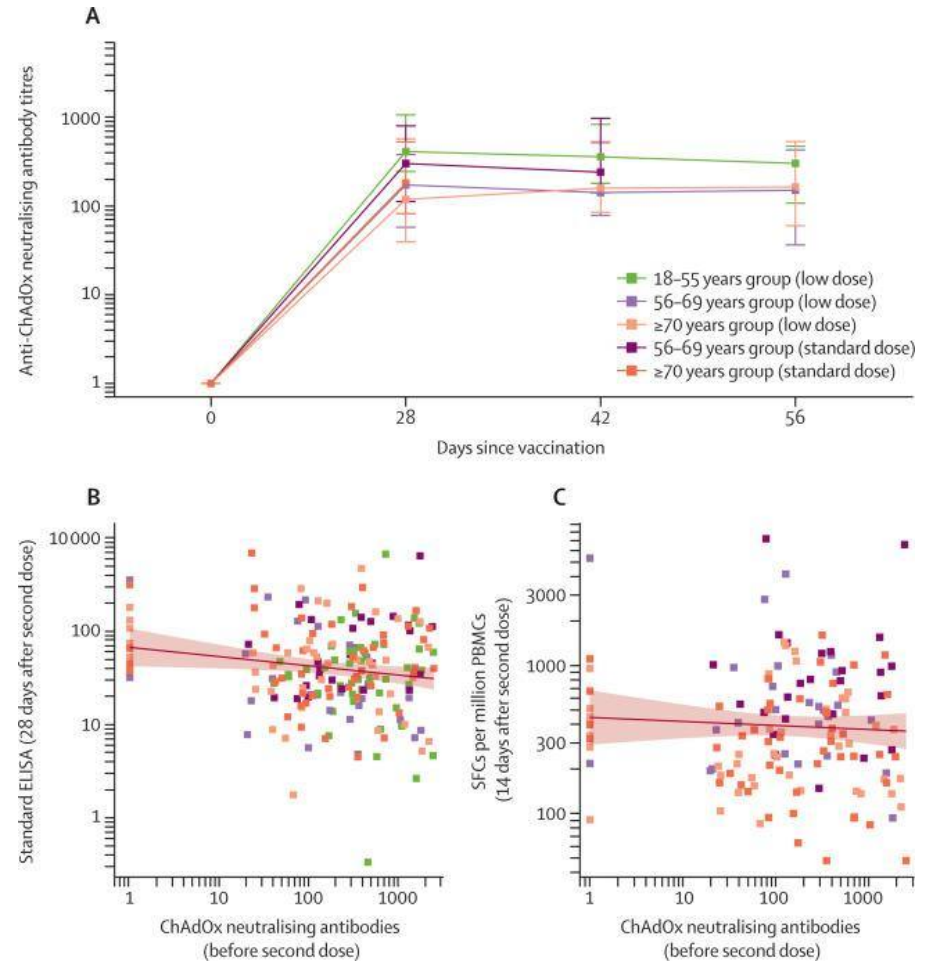
Viral vector vaccines

AstraZeneca/ Johnson & Johnson (Janssen)/
Sputnik V

ChAdOx1 nCoV-19



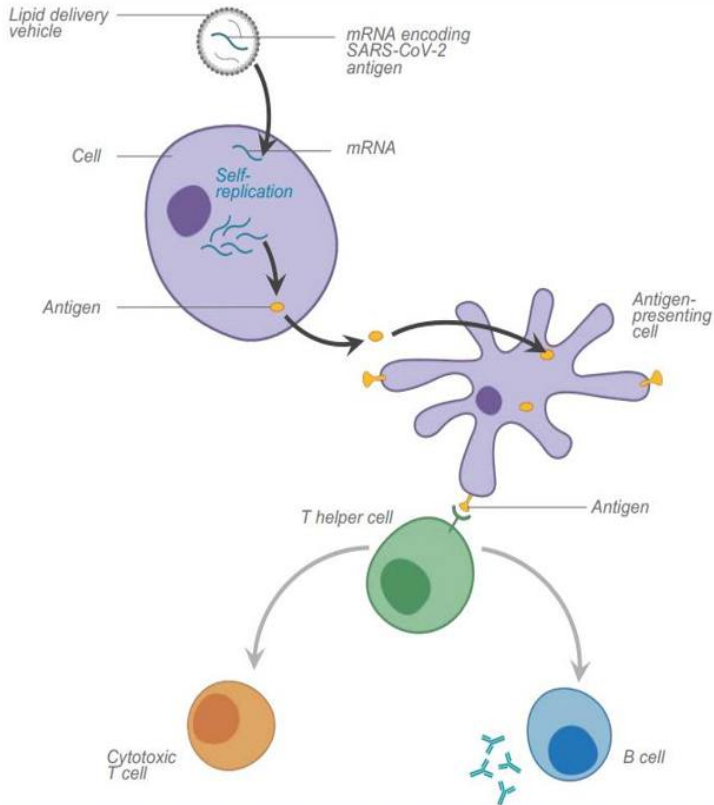
Anti-ChAdOx1 vector after prime and boost doses of vaccine



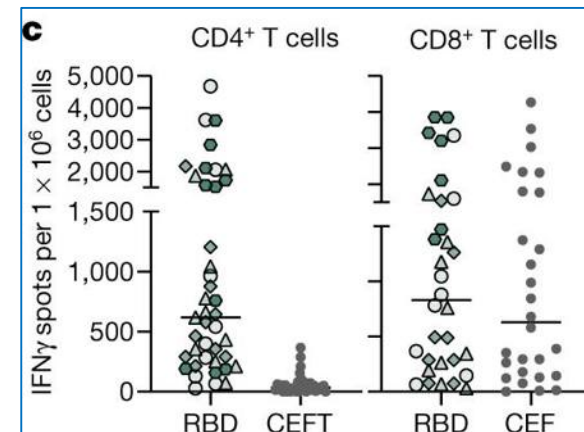
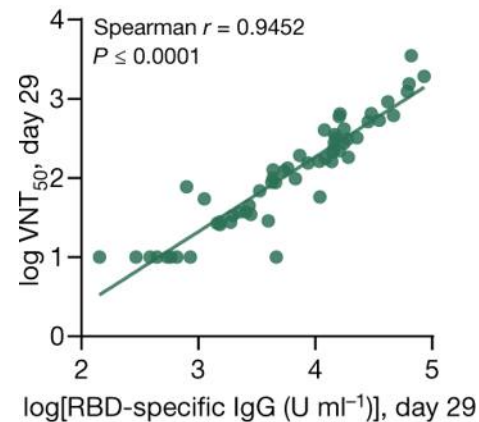
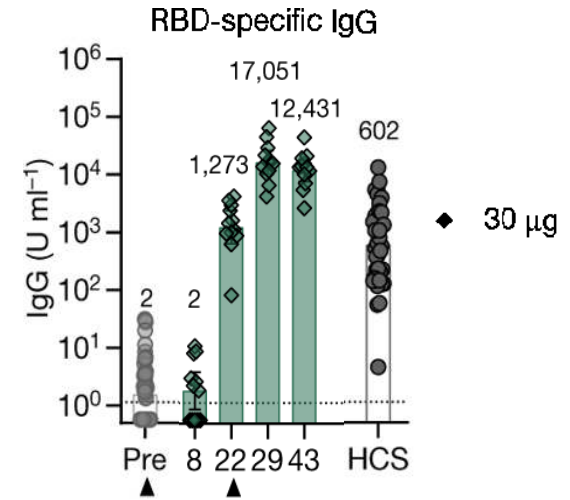
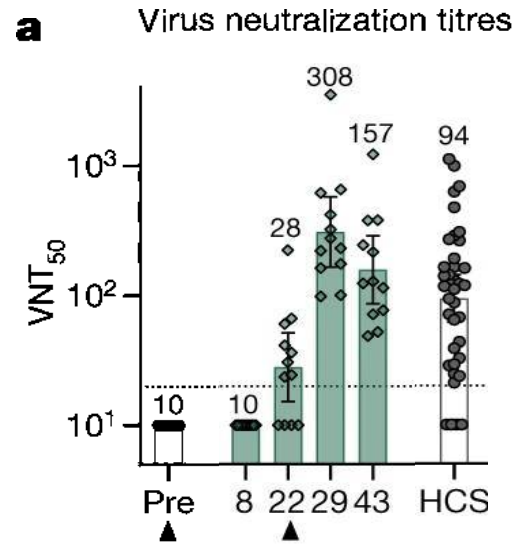
An immune response is induced by vaccine

mRNA vaccines

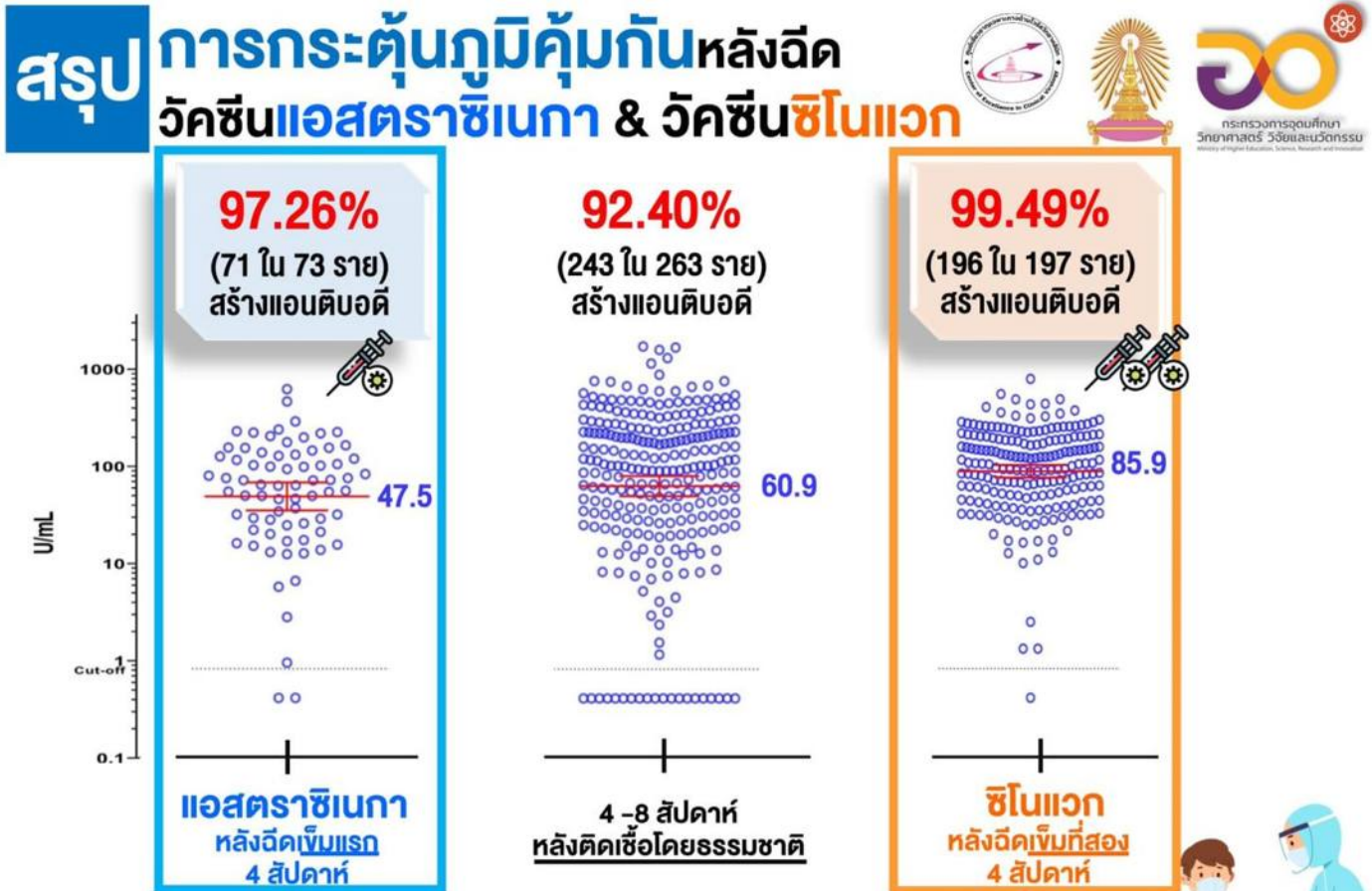
Moderna/Pfizer-BioNtech



COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses



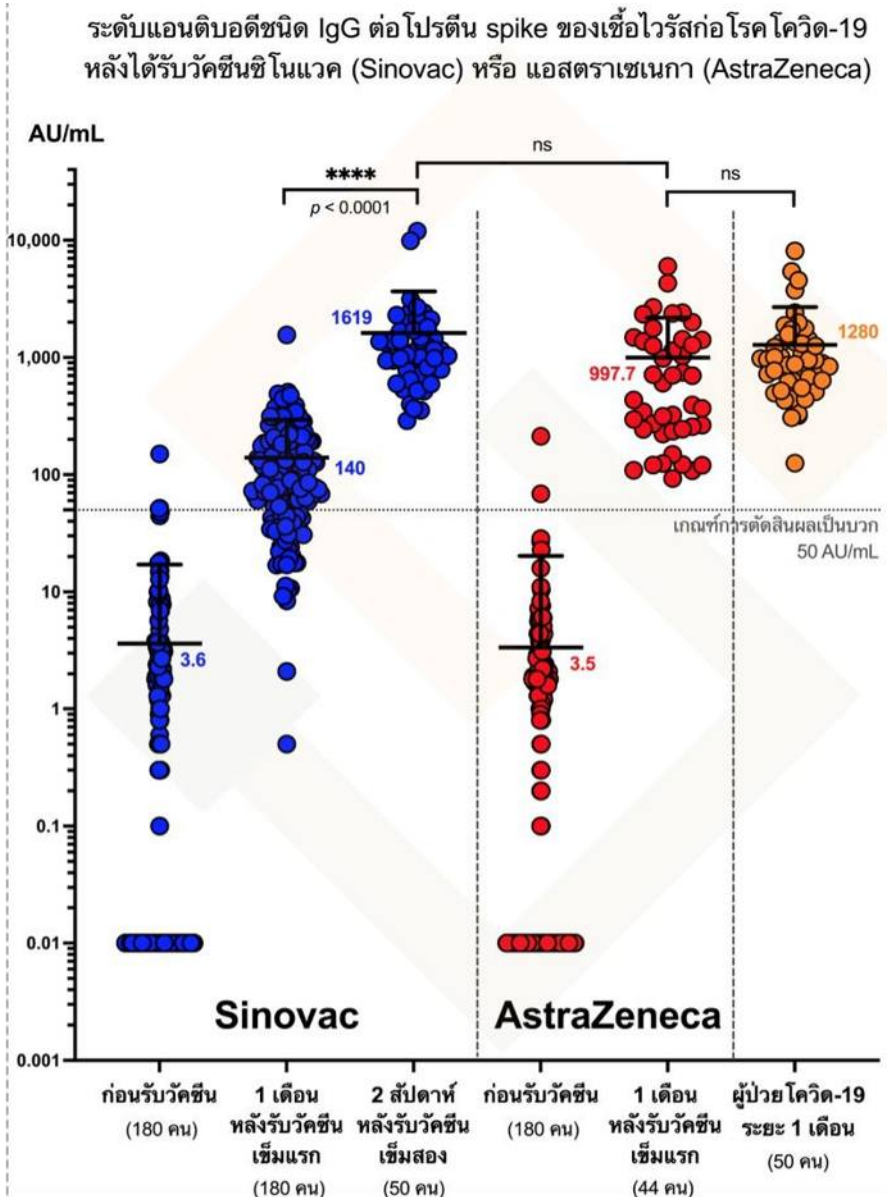
Anti Spike response after Sinovac and AstraZeneca vaccination in Thai population



แหล่งข้อมูล ศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสวิทยาคลินิก คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
สนับสนุนทุนวิจัยโดย สำนักงานการวิจัยแห่งชาติ (วช.) อว. และบริษัท เอ็มเค เรสโตรองด์

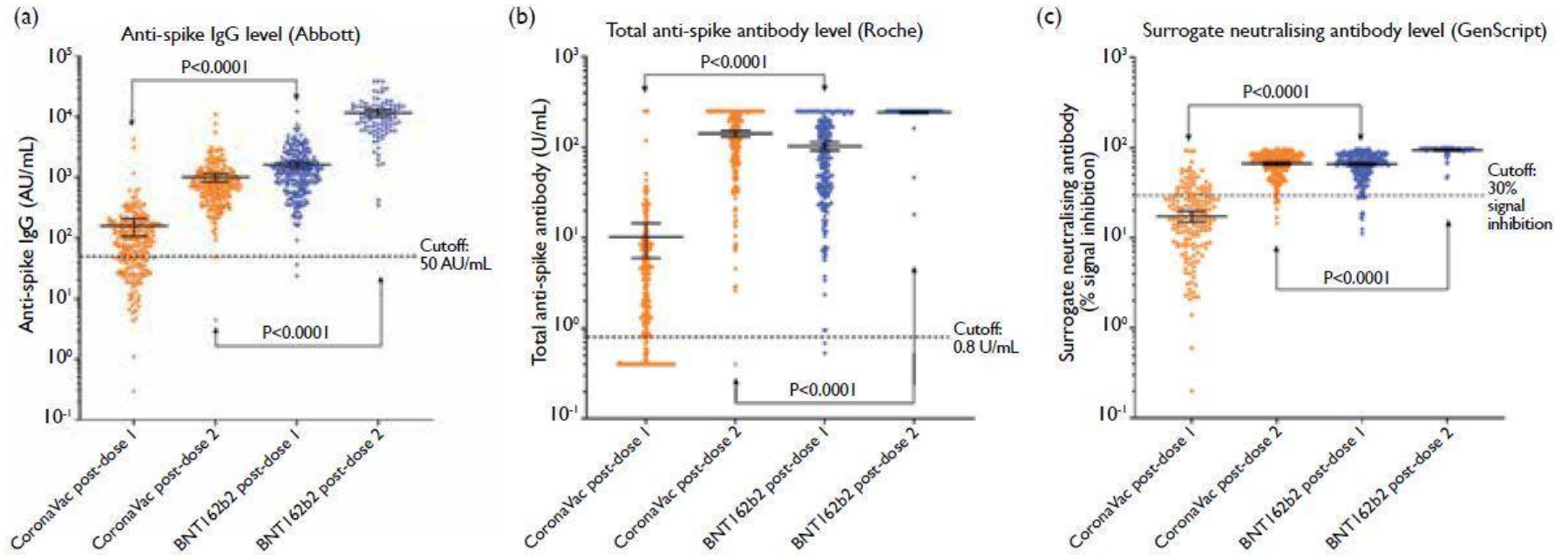


Anti Spike response after Sinovac and AstraZeneca vaccination in Thai population

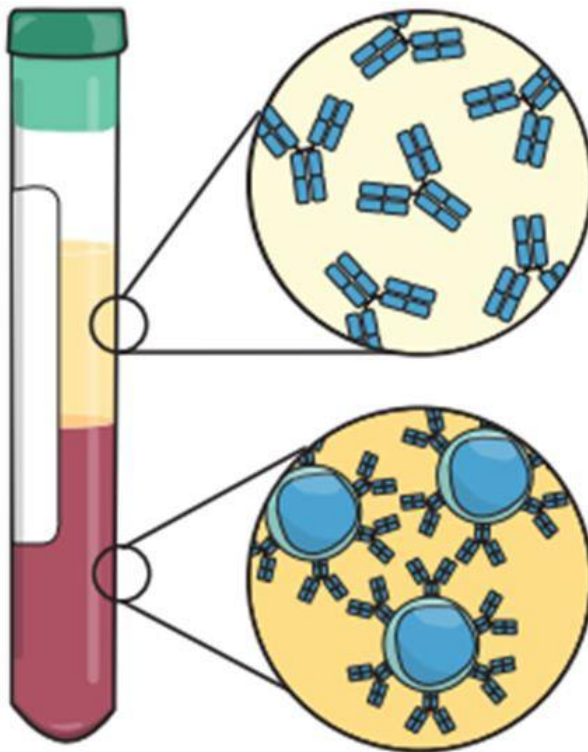


(ภาพจากศูนย์วิจัยคลินิก คณะแพทยศาสตร์ศิริราชพยาบาล : โครงการวิจัยการตอบสนองทางภูมิคุ้มกันและความปลอดภัยหลังได้รับวัคซีน COVID -19 ในบุคลากรการแพทย์ ภายในโรงพยาบาลศิริราช กรุงเทพมหานคร)

Antibody response after Sinovac and Pfizer vaccination in Hongkong



Immunity after infection or vaccination



<https://www.pennmedicine.org/news/releases/2021/april/penn-study-suggests-those-who-had-covid19-may-only-need-one-vaccine-dose>

Serology **Antibody detection**

Presence of Ab indicates past infection or vaccination of an individual.

Cellular memory

Antibody detection



Binding antibody assay

- Detect IgM, IgG, IgA, or total Ig
- Recombinant N and/or S1 proteins are used as the test Ag
- Use purified proteins of virus, not live virus, and can be performed in BSL-2

☼ Immunochromatography

☼ ELISA

☼ Chemiluminescence assay

Qualitative or quantitative assay

Neutralization assay

- Detect total Igs that direct against the neutralizing epitopes of infectious viruses
- Plaque reduction neutralization (PRNT) assay
- Microneutralization test (MNT)
- Focus reduction neutralization test (FRNT)
- Pseudotyped virus neutralization test (pVNT)
- Surrogate virus neutralization test (sVNT)

Binding vs Neutralizing antibodies



Not all antibodies that bind to virus particle will neutralize virus.



Binding antibody

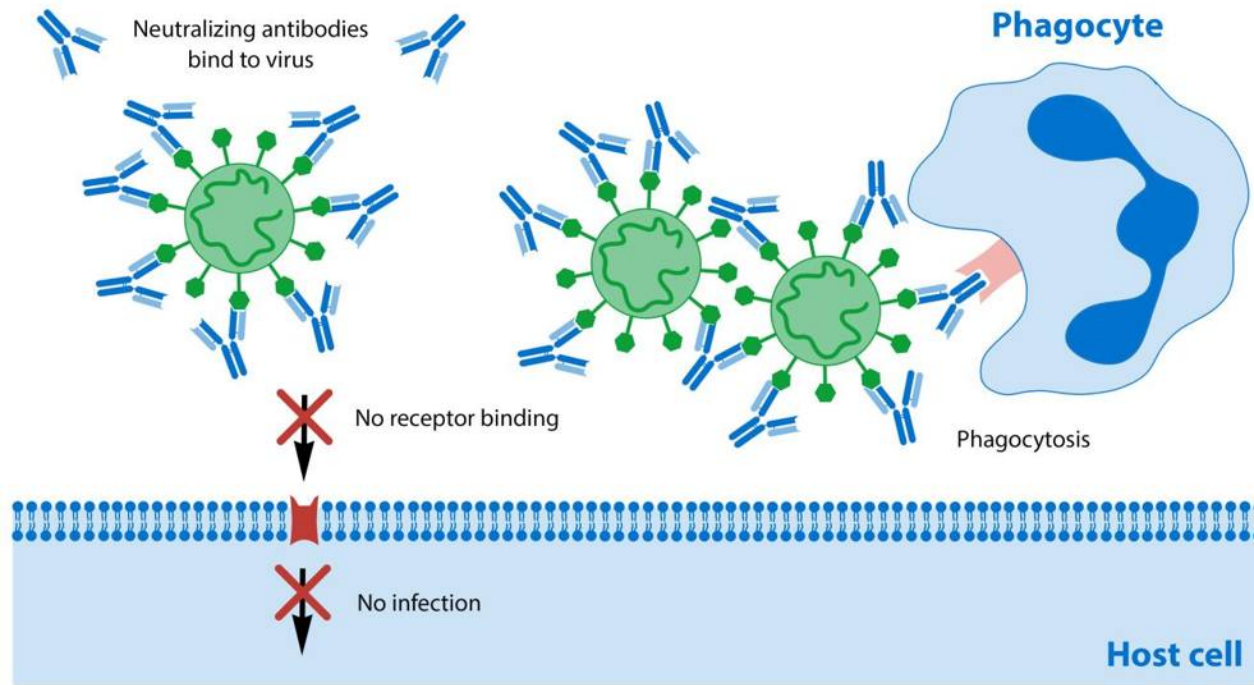
- Bind specifically to virus, but do not inhibit the virus infectivity.
- Not bind to the right region for neutralizing.



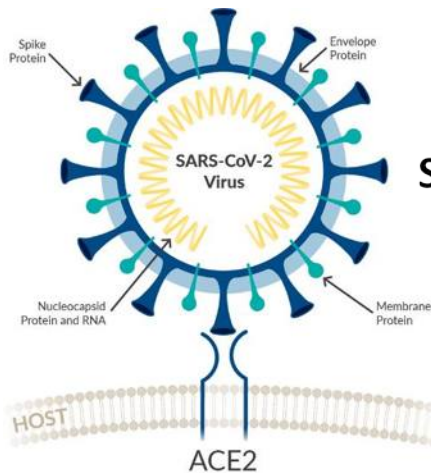
Neutralizing antibody

- Indicate for protective antibody
- Decrease the replication of viruses by blocking attachment, penetration, or uncoating of the virus.
- Neutralization *in vitro* is usually related to protection *in vivo*.

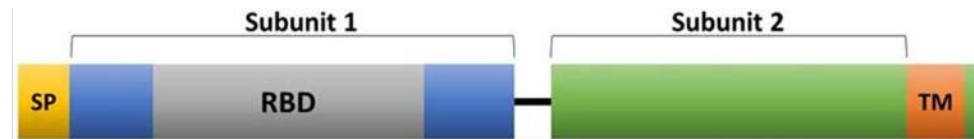
Mechanism of neutralization



<https://www.fluidic.com/resources/What-are-neutralizing-antibodies/>



Spike protein



The neutralizing epitopes in S protein

- RBD (the major site)
 - N-terminal domain
 - S2 subunit
-] S1 subunit

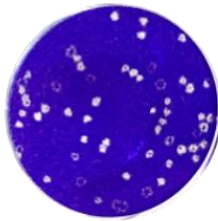


Plaque reduction neutralization test (PRNT)

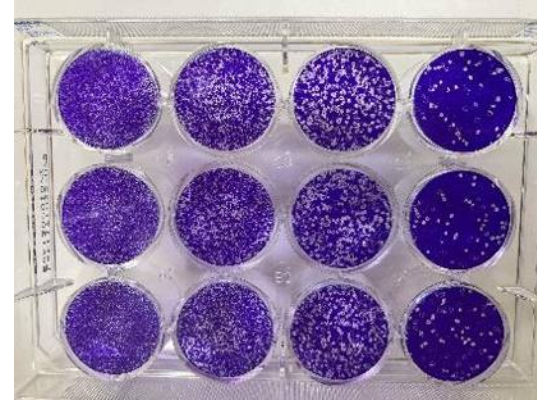
Gold standard method



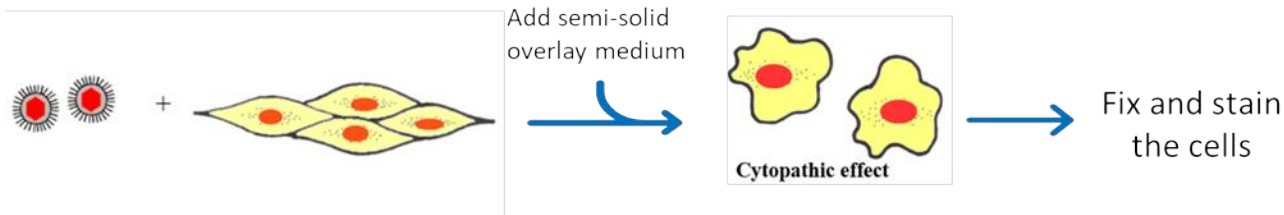
Vero cell control



SARS-CoV-2 plaques

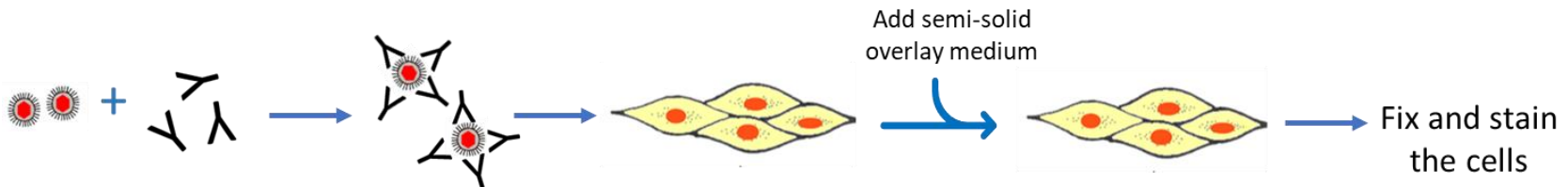
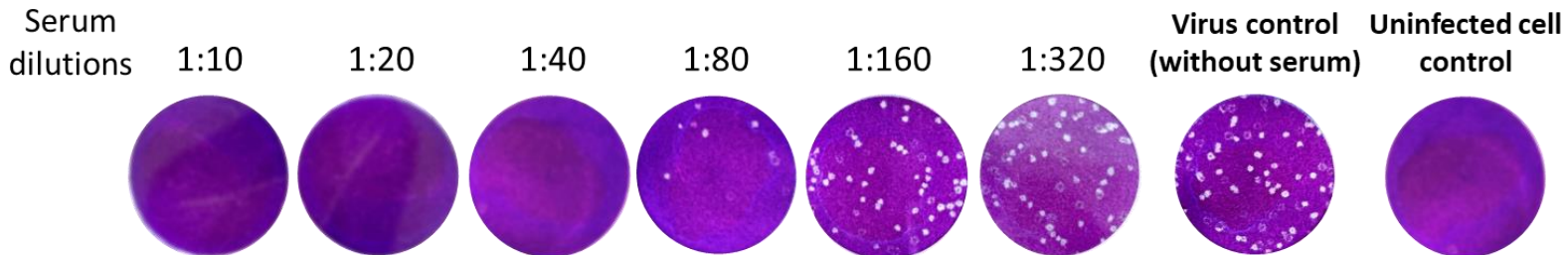


(Jarunee Prasertsopon)



Cut-off point as PRNT50/PRNT90

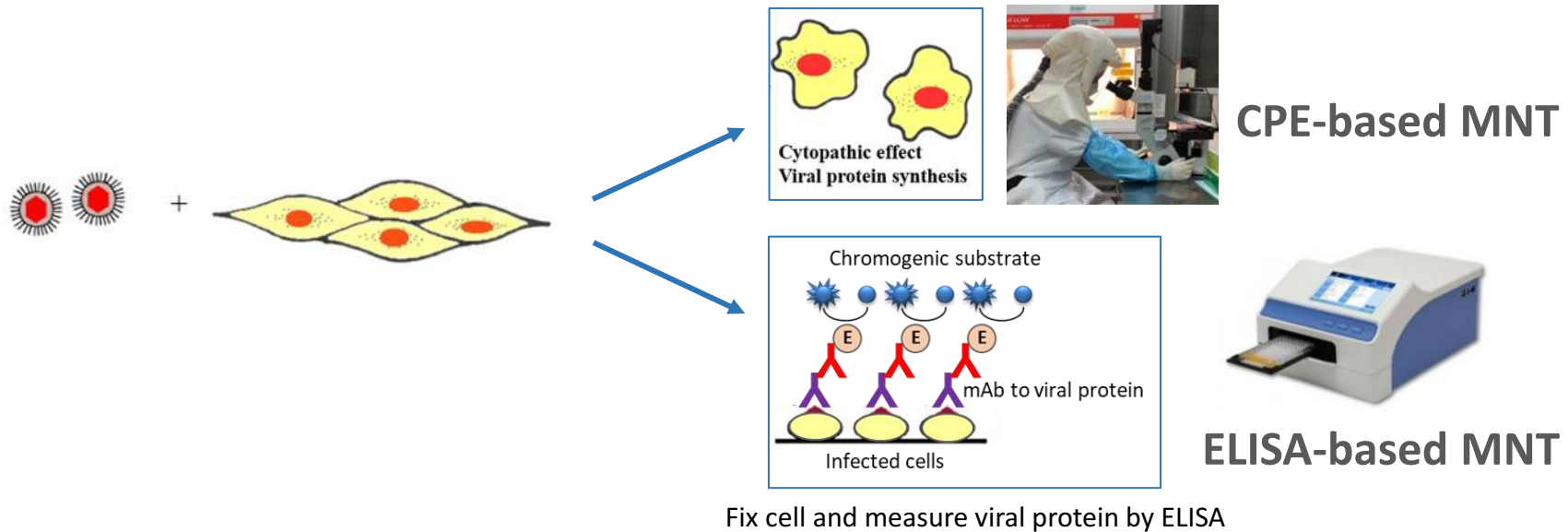
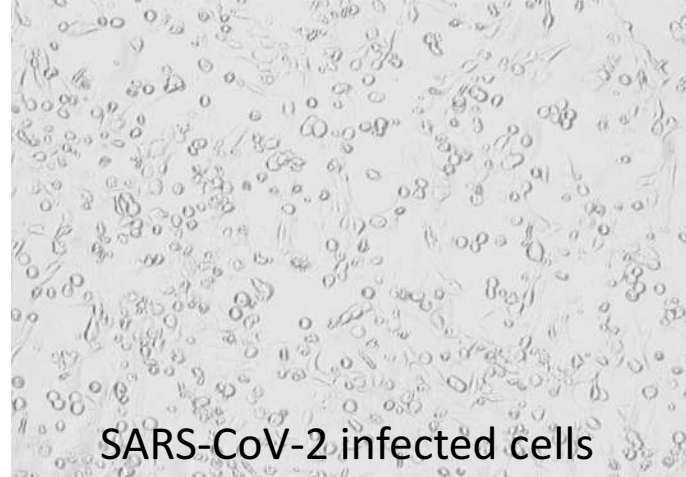
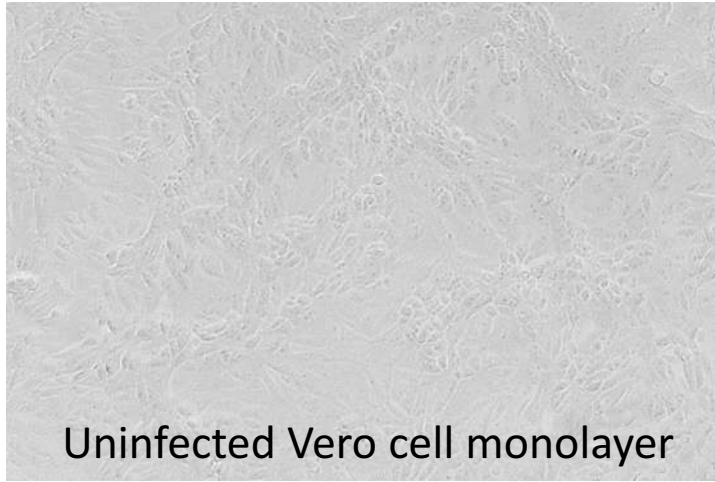
Antibody level





Microneutralization test (MNT)

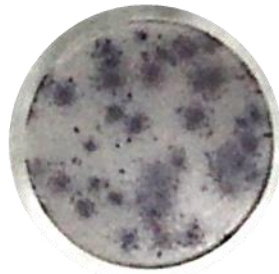
Cytopathic effect (CPE) of SARS-CoV-2 in Vero cell monolayers



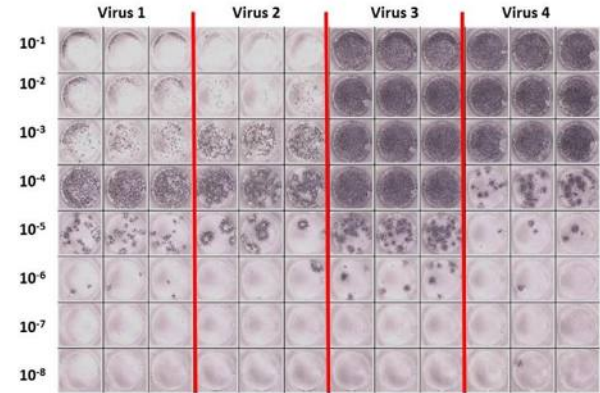
Focus reduction neutralization test (FRNT)



Uninfected cells



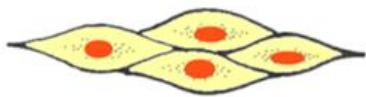
Viral protein synthesis in the infected cells



(Dr. Naruthai Onsirisakul)



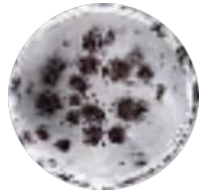
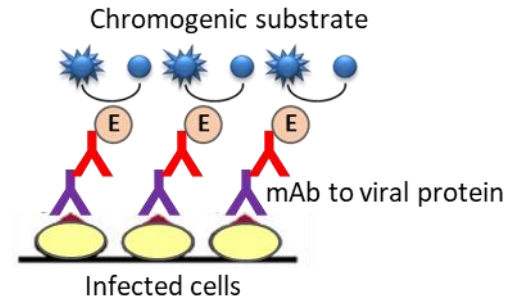
+



Add semi-solid overlay medium

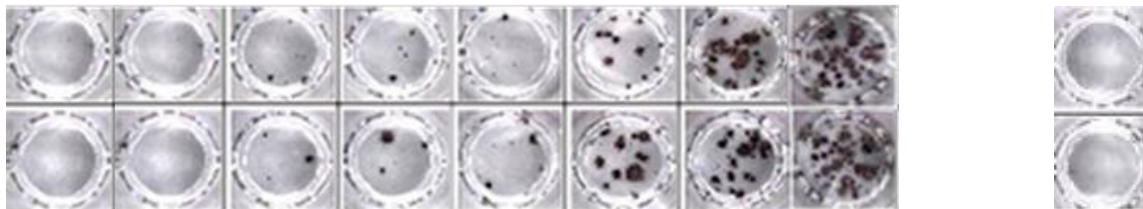


Fixing and immunostaining

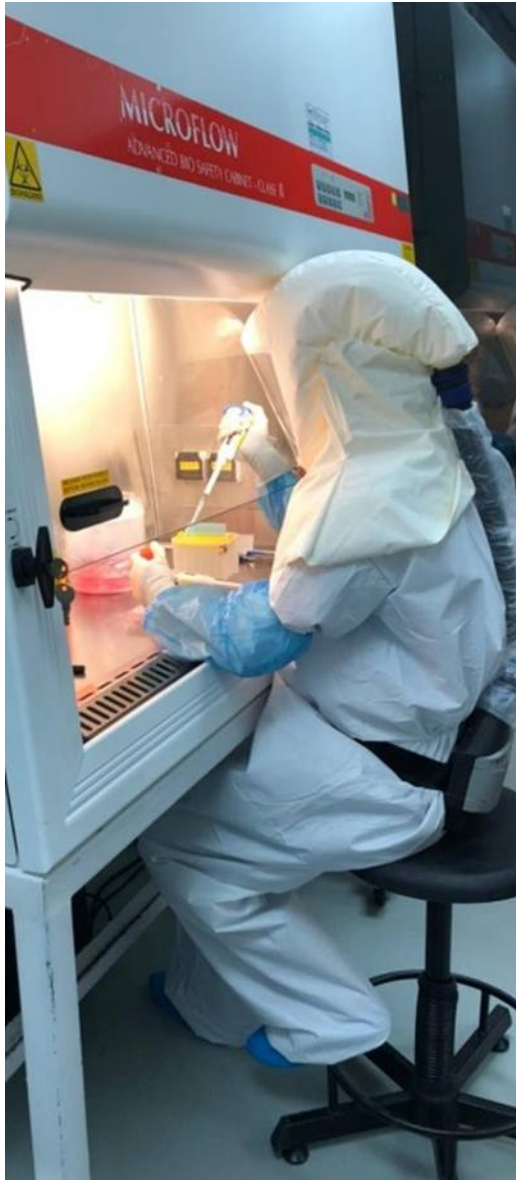


Antibody level

Serum dilutions 1:10 1:20 1:40 1:80 1:160 1:320 1:640 Virus control Uninfected cell



BSL-3 facilities are required for PRNT, FRNT, or MNT




(BSL-3 facilities at Faculty of Veterinary Science, Mahidol University)



Alternative assays to PRNT, FRNT, and MNT

- Avoid usage of risk group 3 virus
- Easy to handle
- Less time consume
- Less laborious

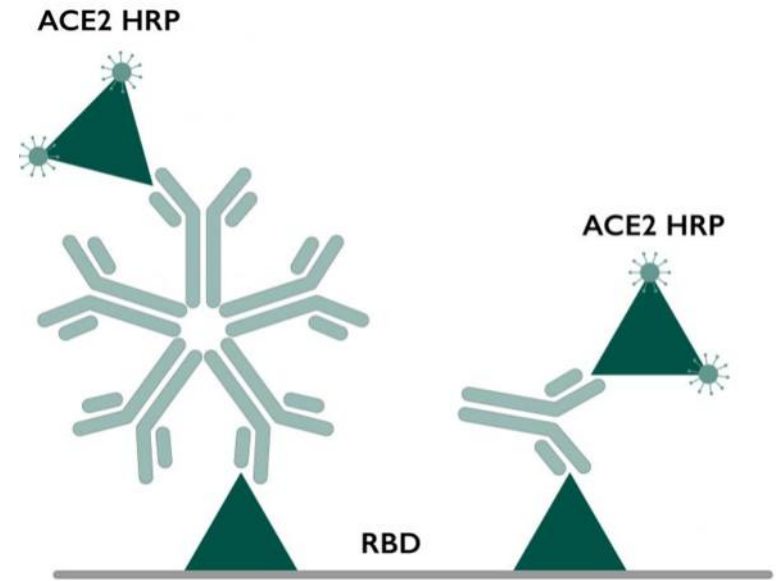
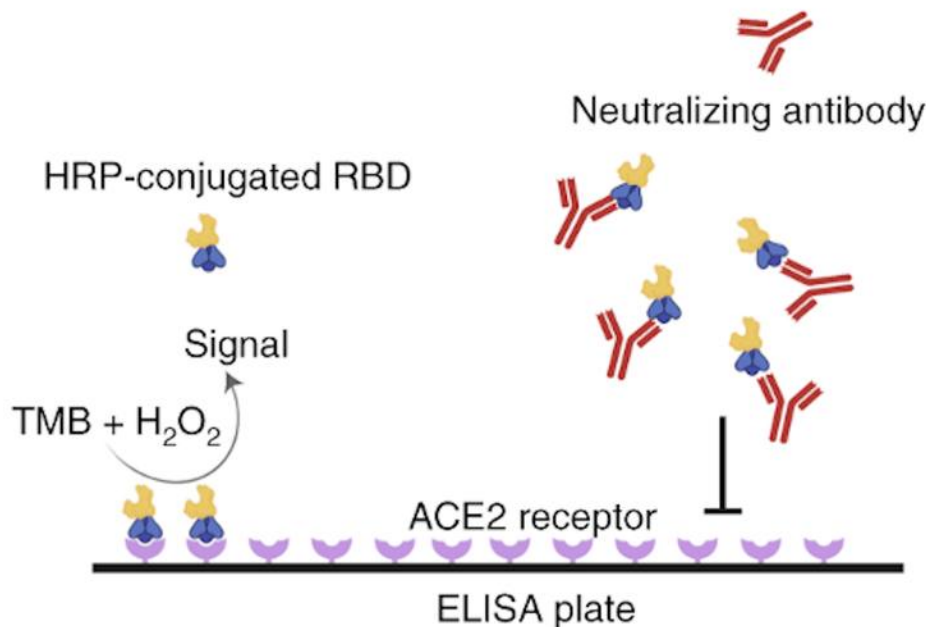
 **Surrogate virus Neutralization assay**

 **Pseudotyped virus-neutralization assay**



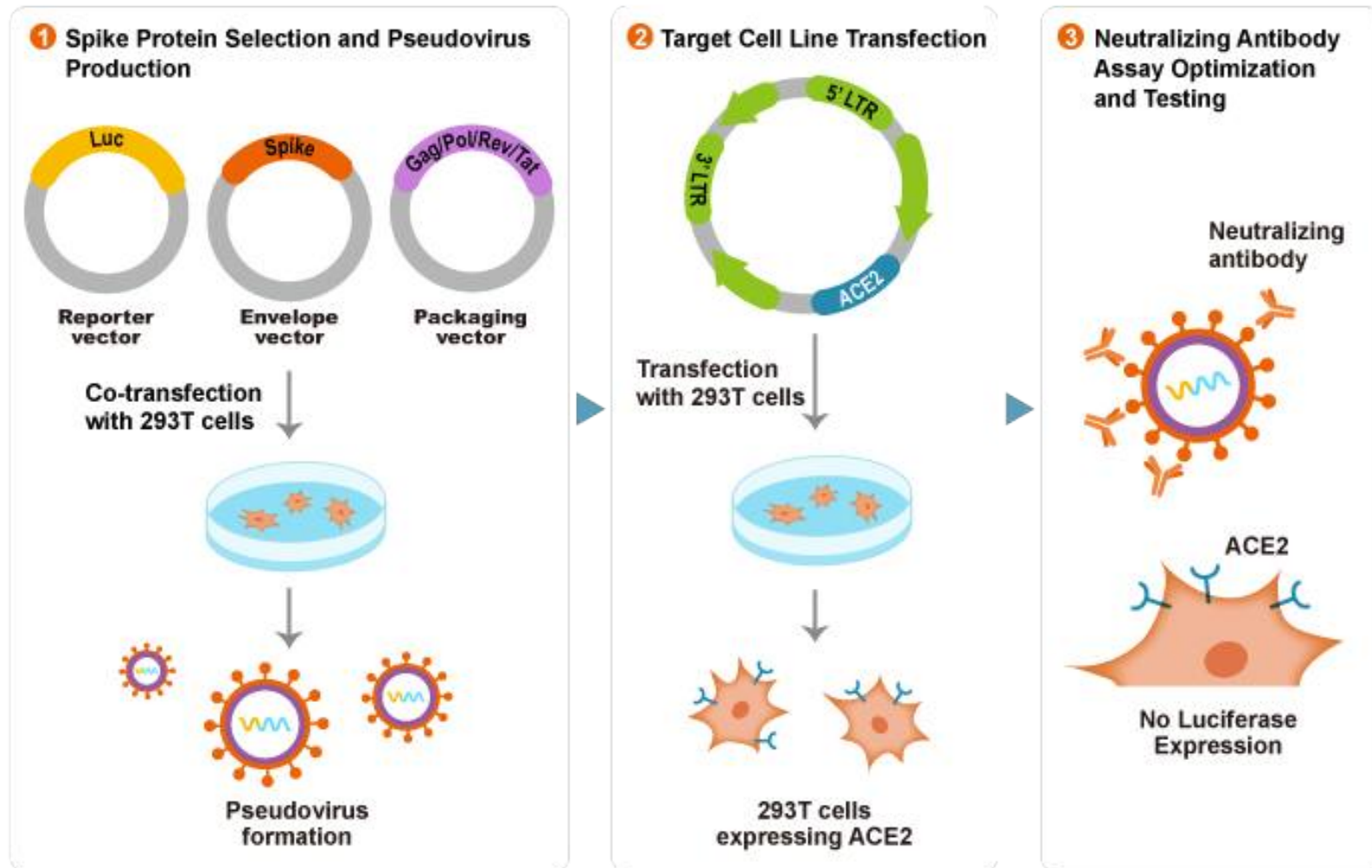
Surrogate virus Neutralization test (sVNT)

- Cell-free neutralization assay
- Measurement the circulating antibodies against SARS-CoV-2 that block interaction between the S (RBD) protein with the human ACE2 cell surface receptor



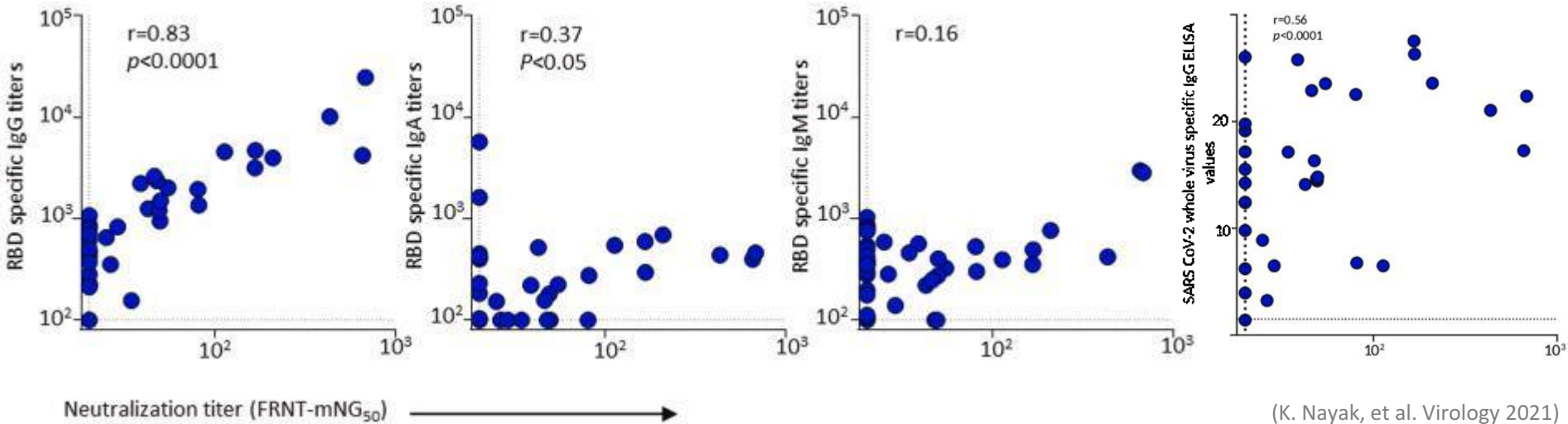
Pseudotyped virus neutralization test (pVNT)

- Need cell culture system
- Pseudotyped virus cannot replicate.

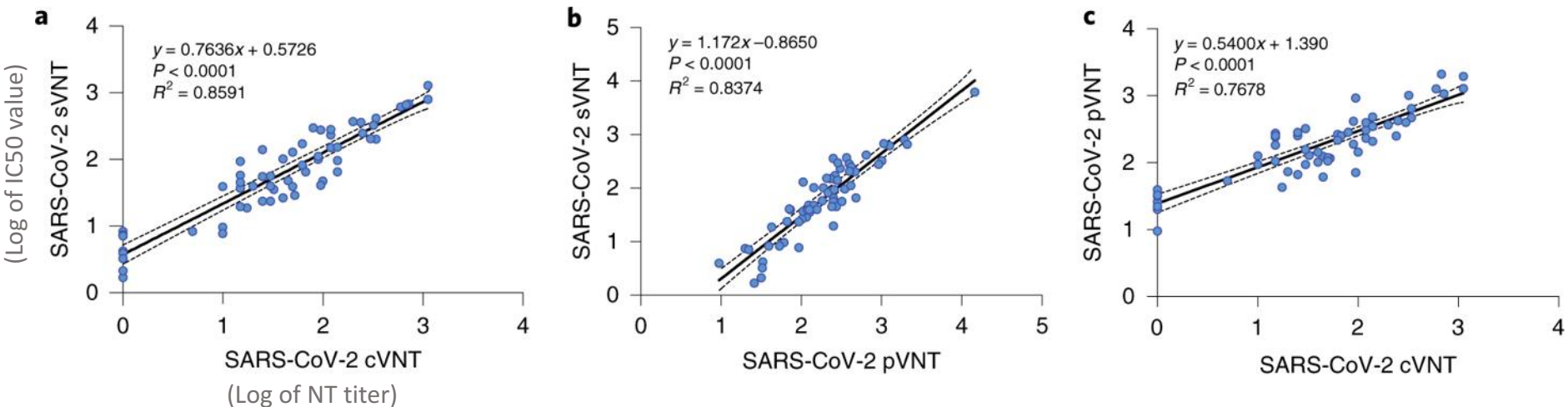




Correlation between RBD specific Ab titer and NT titer



Correlation for SARS-CoV-2 antibody by sVNT, pVNT, and cVNT



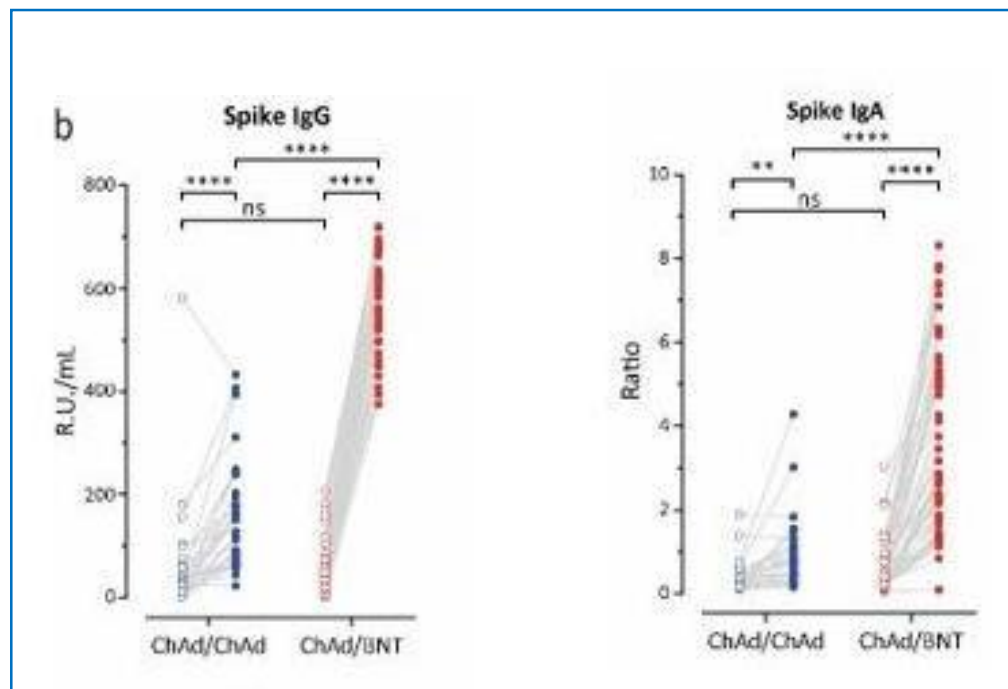
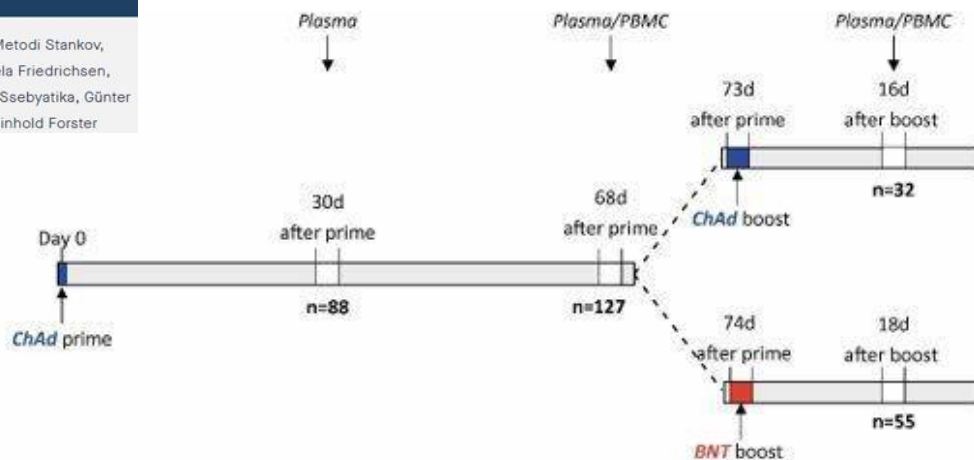
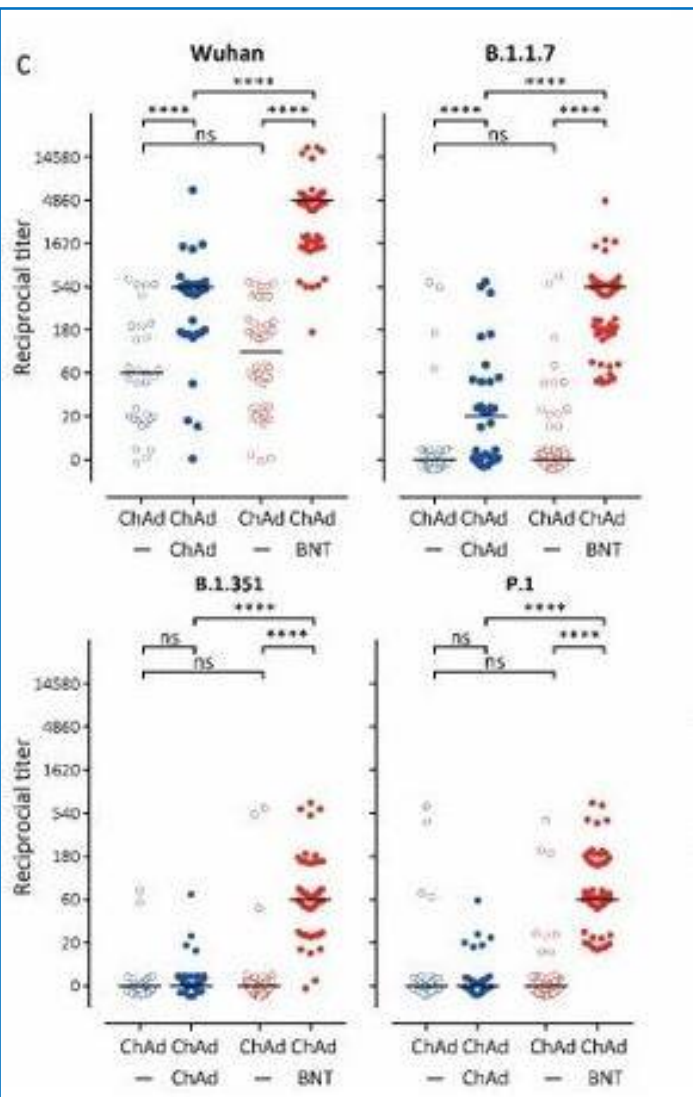
Mix-and-match COVID vaccines trigger stronger immune responses?

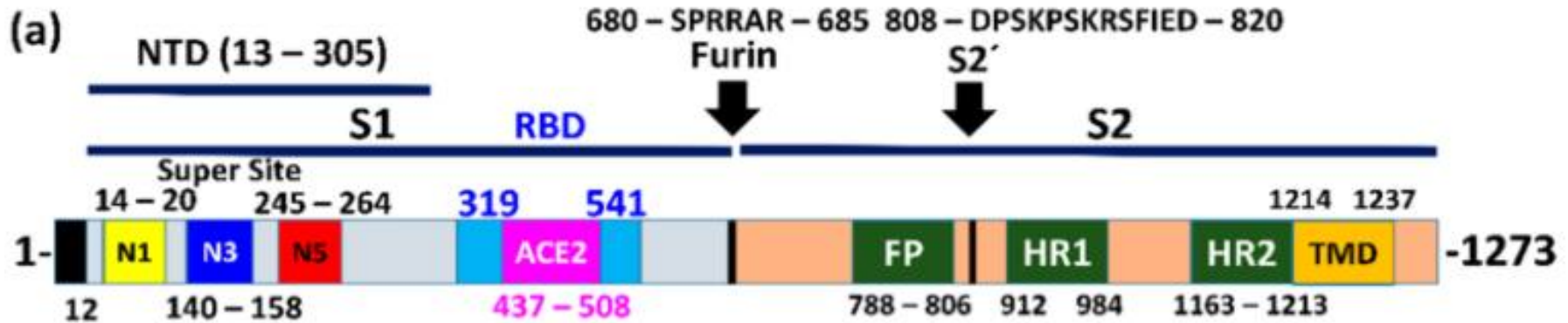
Homologous / Heterologous prime boost vaccination



Humoral and cellular immune response against SARS-CoV-2 variants following heterologous and homologous ChAdOx1 nCoV-19/BNT162b2 vaccination.

> Georg Behrens, Joana Barros-Martins, Swantje Hammerschmidt, Anne Cossmann, Ivan Odak, Metodi Stankov, Gema Morillas Ramos, Alexandra Dopfer-Jablonka, Annika Heidemann, Christiane Ritter, Michaela Friedrichsen, Christian Schultze-Florey, Inga Ravens, Anja Bubke, Jasmin Ristenpart, Anika Janssen, George Seebaytika, Günter Schmidt, Jan Münch, Markus Hoffmann, Stefan Pöhlmann, Thomas Krey, Berislav Bosnjak, Reinhold Forster





Receptor binding motif

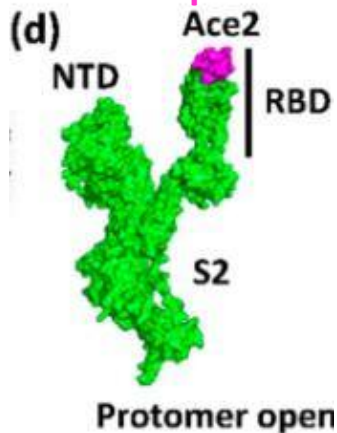
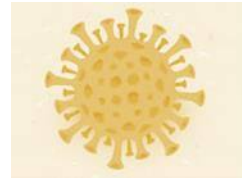
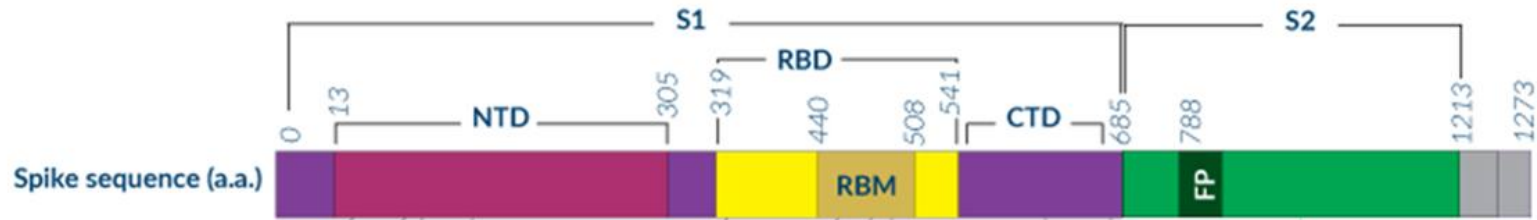


Figure 1. Domain Organization of the SARS-CoV-2 spike protein and structural features. (a) Domain organization of spike. Signal peptide: 1–12aa; S1-domain: 13–685aa; S2-domain: 686–1273aa; N-terminal domain (NTD): 13–305aa; Supersite loops: N1:14–20aa; N3: 140–158aa; N5: 245–264aa; Receptor binding domain (RBD): 319–541aa; Receptor binding motif (ACE2): 437–508aa; Furin cleavage sequence: 680–685aa; Fusion peptide (FP): 788–806aa; Heptad repeat region 1 (HR1): 912–984aa; Heptad repeat region 2 (HR2): 1163–1213aa; Transmembrane domain (TMD): 1214–1237aa; Cytoplasmic domain: 1238–1273aa; (b) Surface of the closed spike trimer (PDB: 6VXX); (c) Surface of the open spike trimer—one protomer open (green) (PDB: 6VYB); (d) Open protomer from (c) with the Ace2 binding motif (magenta) and the RBD both indicated; (e) Surface of the open protomer with the three loops of the N-terminal supersite (N1: yellow; N3: blue; N5: red) and the Ace2 binding site (magenta) highlighted (PDB: 7DF4);

RBD = 319-541 amino acids

ACE2 (RBM) = 437-508 amino acids
440-508

SARS-CoV-2 variants of concern



Wuhan-Hu-1

**Alpha: B.1.1.7
U.K. variant**

**Beta: B.1.351
S.A. variant**

**Gamma: P.1
Brazil variant**

**Delta: B.1.617.2
India variant**

D614

Δ69/70
Δ144/145
N501Y
A570D
D614G
P681H
T716I
S982A
D1118H
E484K*
S494P*

D80A
D215G
Δ241-243
K417N
E484K
N501Y
D614G
A701V

L18F
T20N
P26S
D138Y
R190S
K417T
E484K
N501Y
D614G
H655Y
T1027I

T19R
G142D
Δ157-158
L452R
T478K
D614G
P681R
D950N

* = detected in some sequences but not all