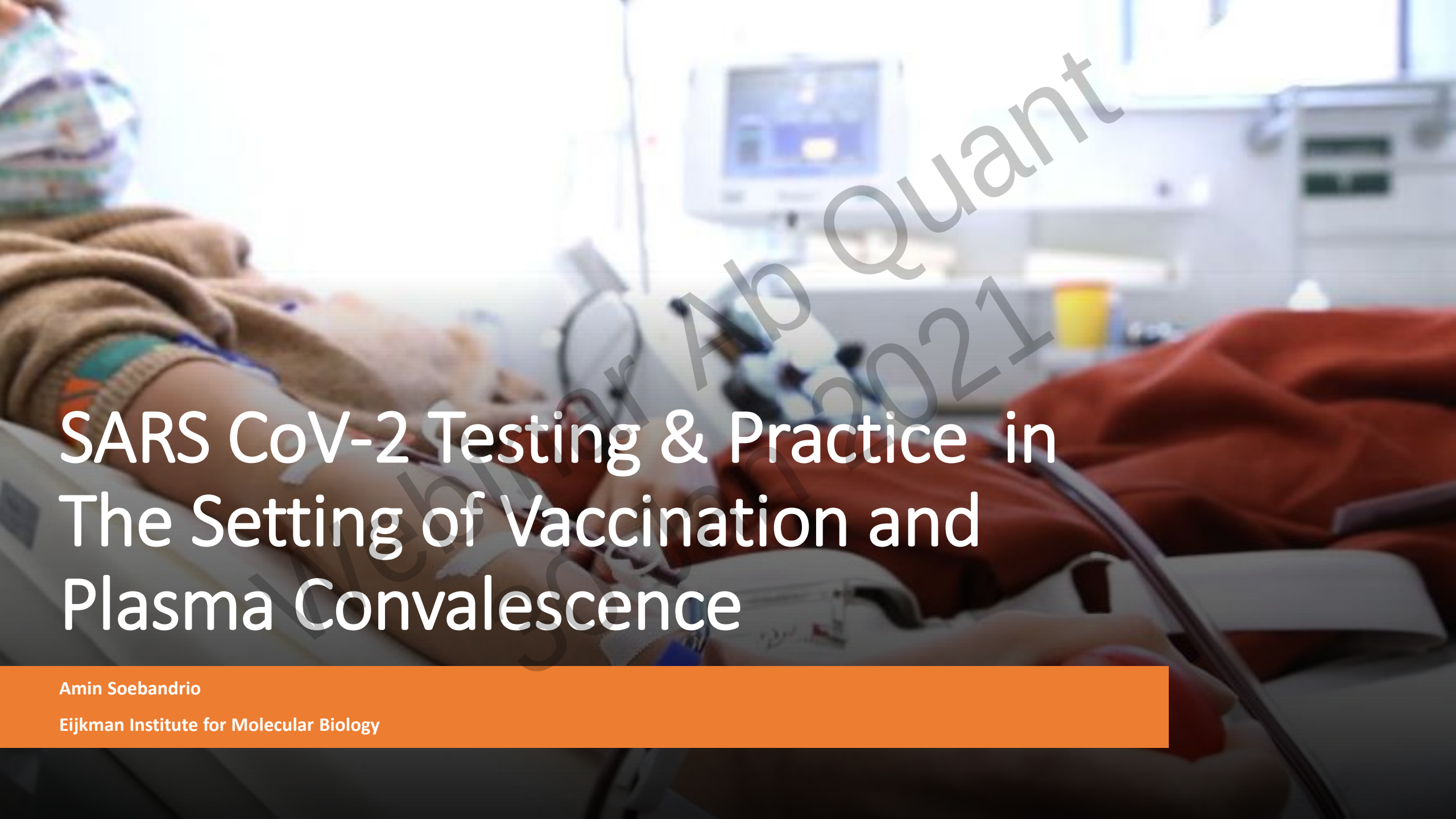


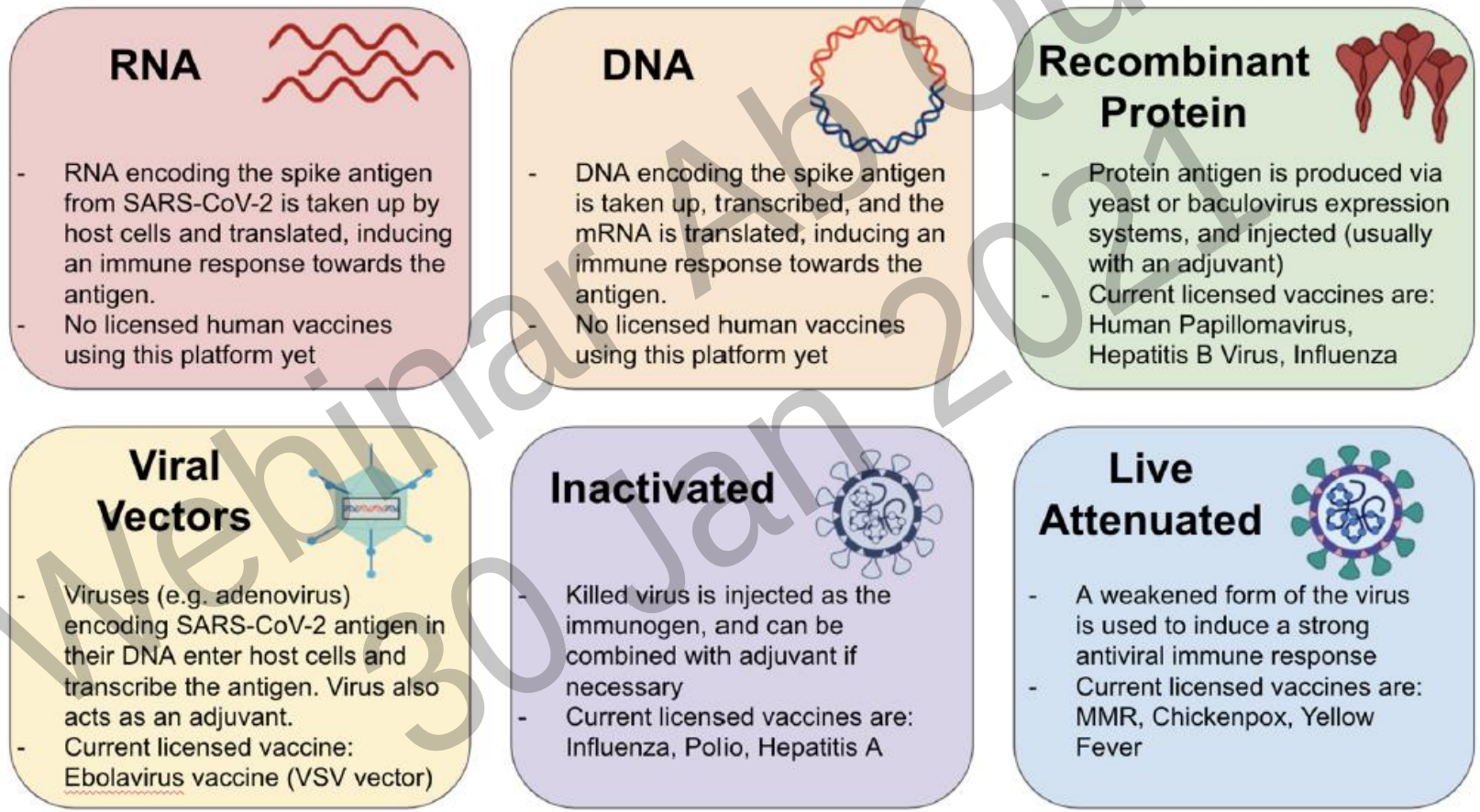
SARS CoV-2 Testing & Practice in The Setting of Vaccination and Plasma Convalescence

Amin Soebandrio

Eijkman Institute for Molecular Biology

- **Program vaksin dan plasma konvalesen(PK) di Indonesia secara umum & program di Indonesia***
  2. Penelitian yang sedang berjalan di Indonesia terkait dengan uji efektivitasnya vaksin dan PK
  ***3. Tes- tes pendukung yang mendukung pengujian vaksin dan PK, difokuskan**
  kepada tes tes SARS CoV 2*
  tidak hanya terfokus pada pengukuran antibodi, tetapi bisa include PCR, PRNT, korelasi klinis dan lainnya
  4. Pentingnya pengukuran kuantitatif antibodi terutama korelasi dengan konsep netralisasi dan PRNT
  ***5. Apabila berkenan, dapat menyebutkan kerja sama penelitian dengan Roche**
  yaitu uji kesesuaian dengan PRNT*

Current vaccine platforms of COVID-19 vaccine candidates

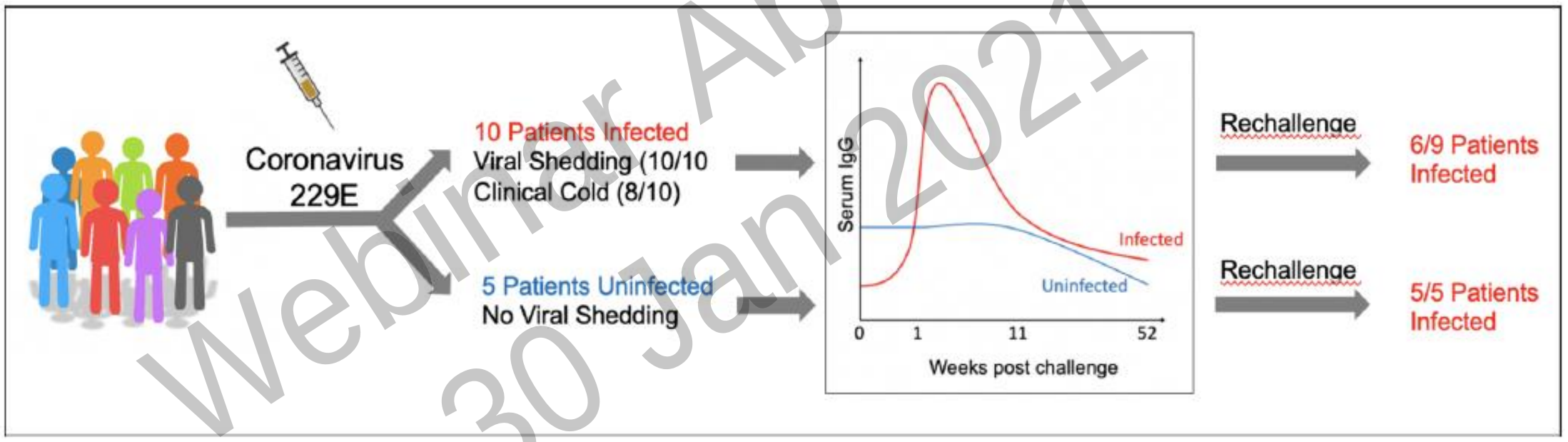


L. Grigoryan and B. Pulendran . Seminars in Immunology 50 (2020) 101422

Could a Blood Test Show if a Covid-19 Vaccine Works?

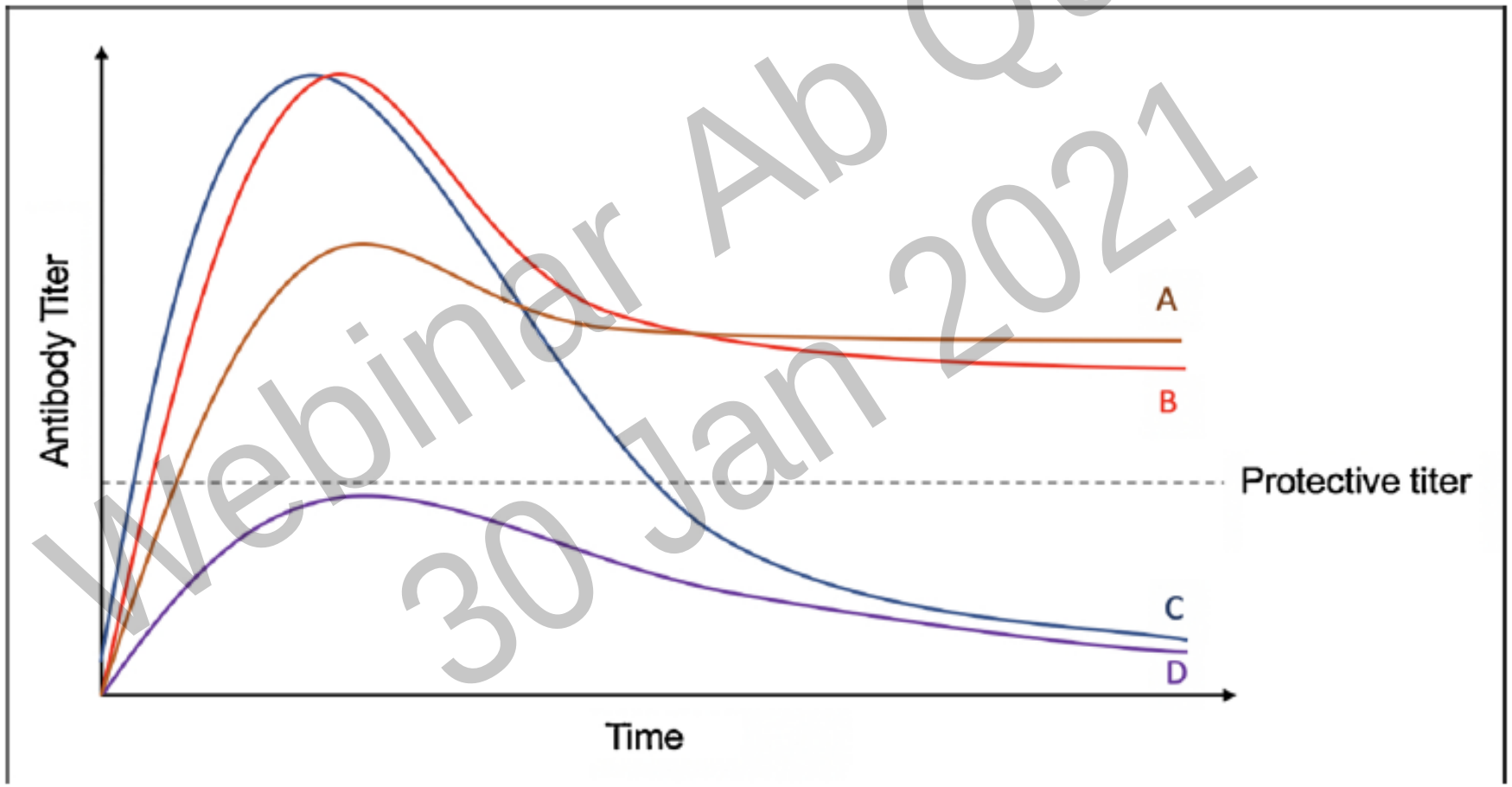
Human challenge study of coronavirus 229E

- 15 volunteers were inoculated with 229E, 10 of whom became infected.
- Chart shows average antibody titers in the infected and infected groups.
- All subjects were rechallenged, and all originally uninfected patients got infected upon secondary exposure, compared to 6 out of the 9 patients who were originally infected.



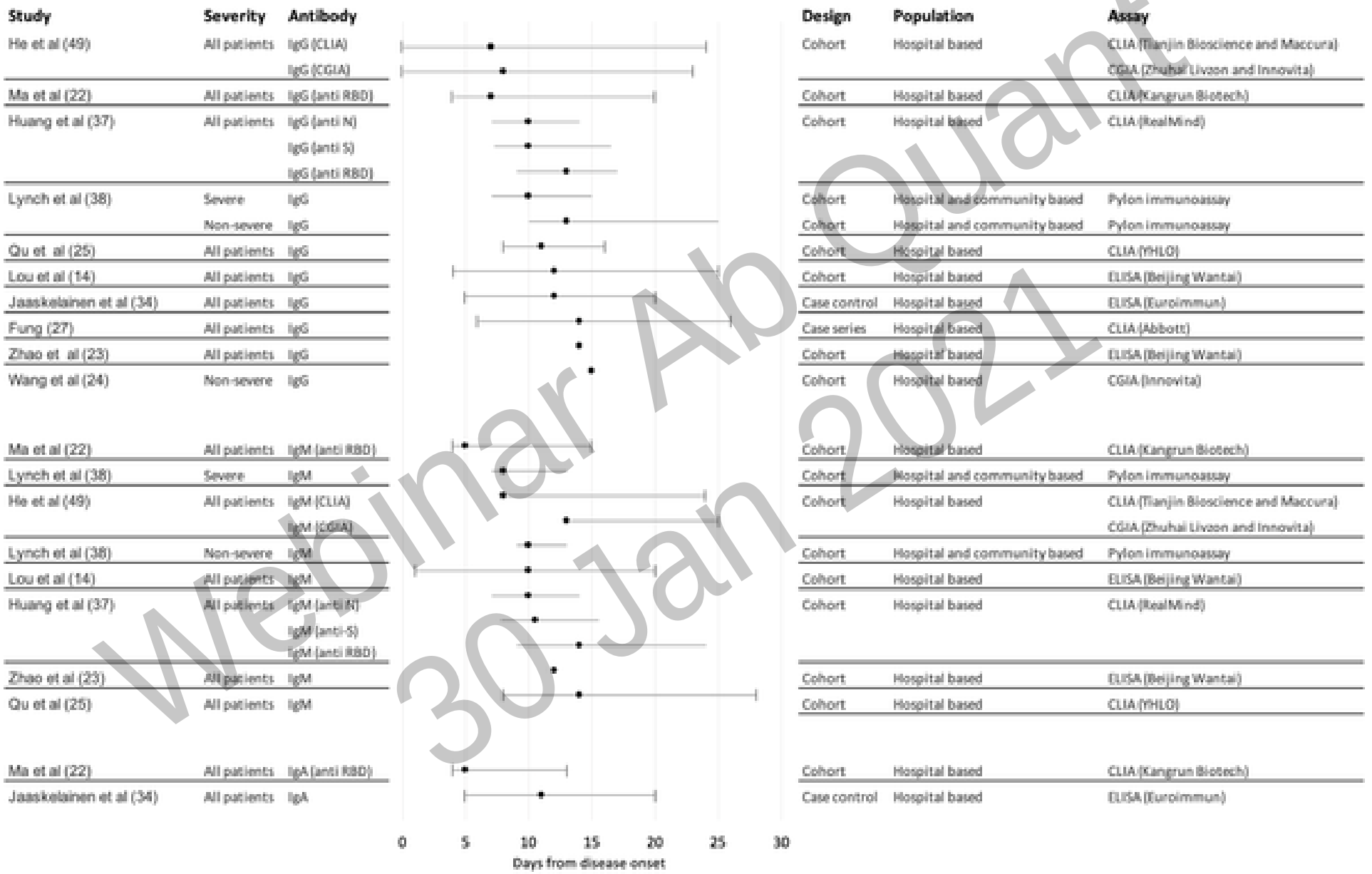
K.A. Callow, et al. Epidemiol. Infect. 105 (2) (1990) 435–446.

The durability of antibody responses over time in 4 different infection/vaccination scenarios



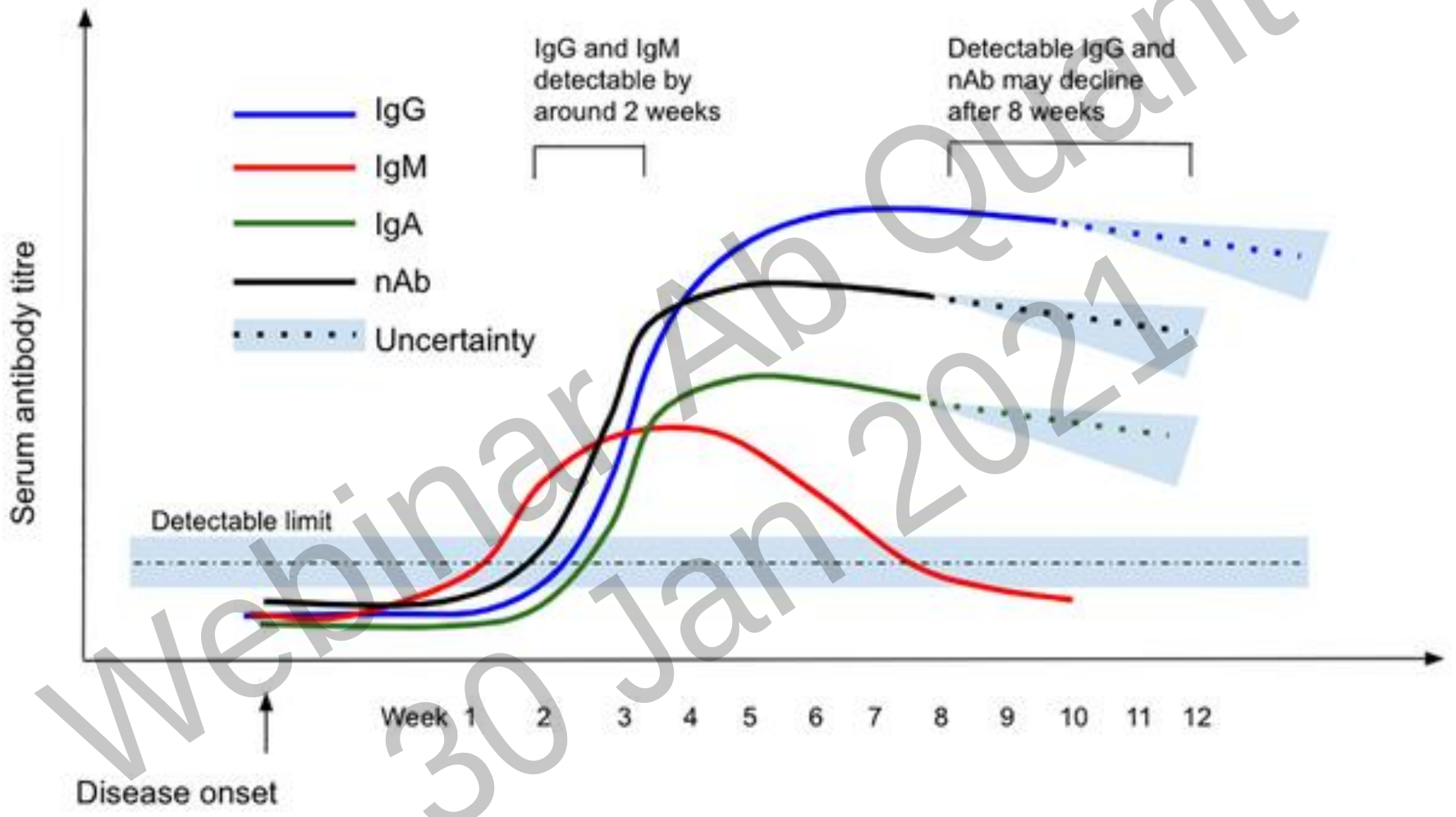
L. Grigoryan and B. Pulendran . Seminars in Immunology 50 (2020) 101422

Fig 2. Forest plot of median time to seroconversion by severity across included studies.



Post N, Eddy D, Huntley C, van Schalkwyk MCI, Shrotri M, et al. (2020) Antibody response to SARS-CoV-2 infection in humans: A systematic review. PLOS ONE 15(12): e0244126. https://doi.org/10.1371/journal.pone.0244126
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0244126

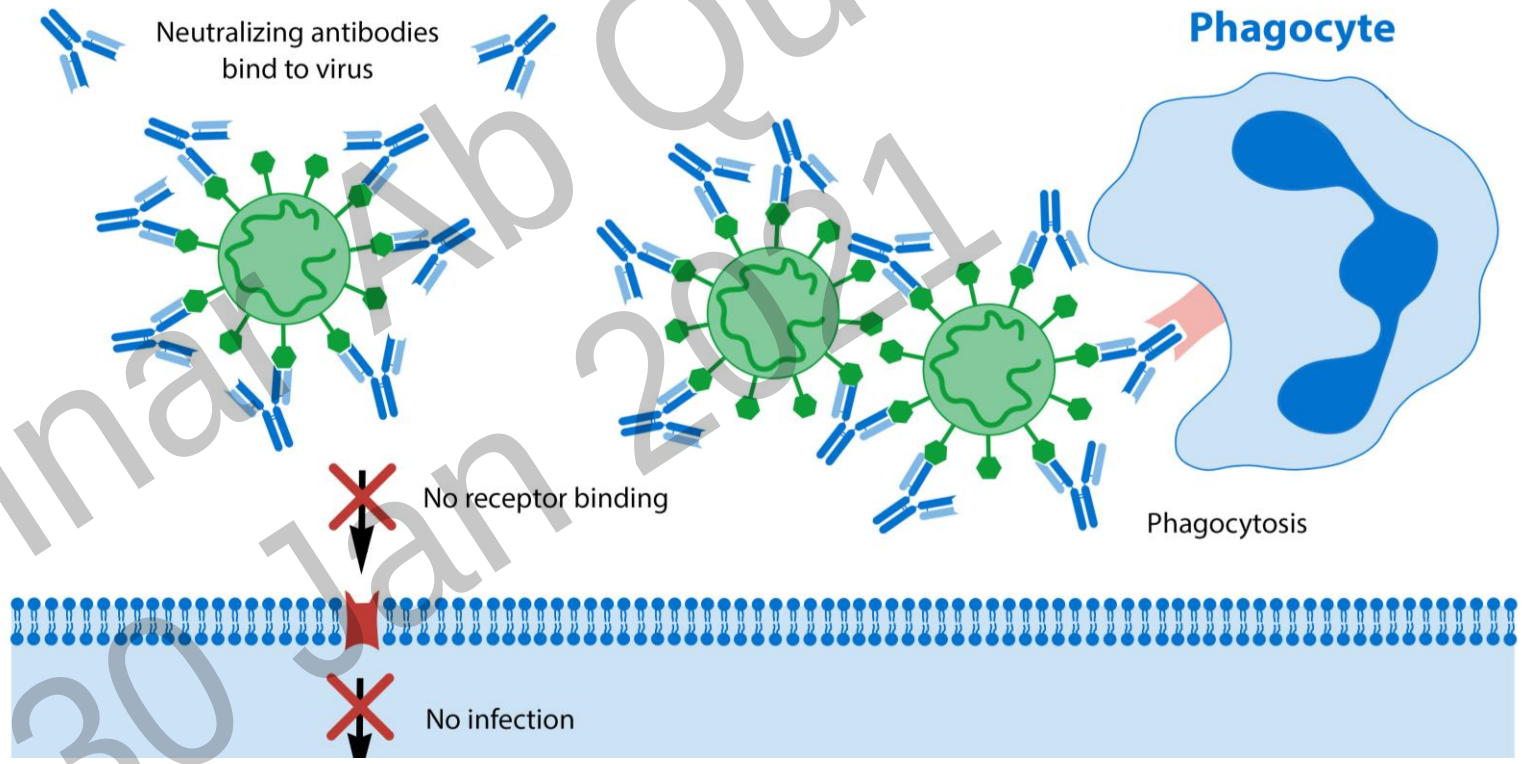
Fig 3. Schematic showing the scale of IgG/IgM/IgA/Neutralising Ab response over time from disease onset.



Post N, Eddy D, Huntley C, van Schalkwyk MCI, Shrotri M, et al. (2020) Antibody response to SARS-CoV-2 infection in humans: A systematic review. PLOS ONE 15(12): e0244126. https://doi.org/10.1371/journal.pone.0244126
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.024

PLOS ONE

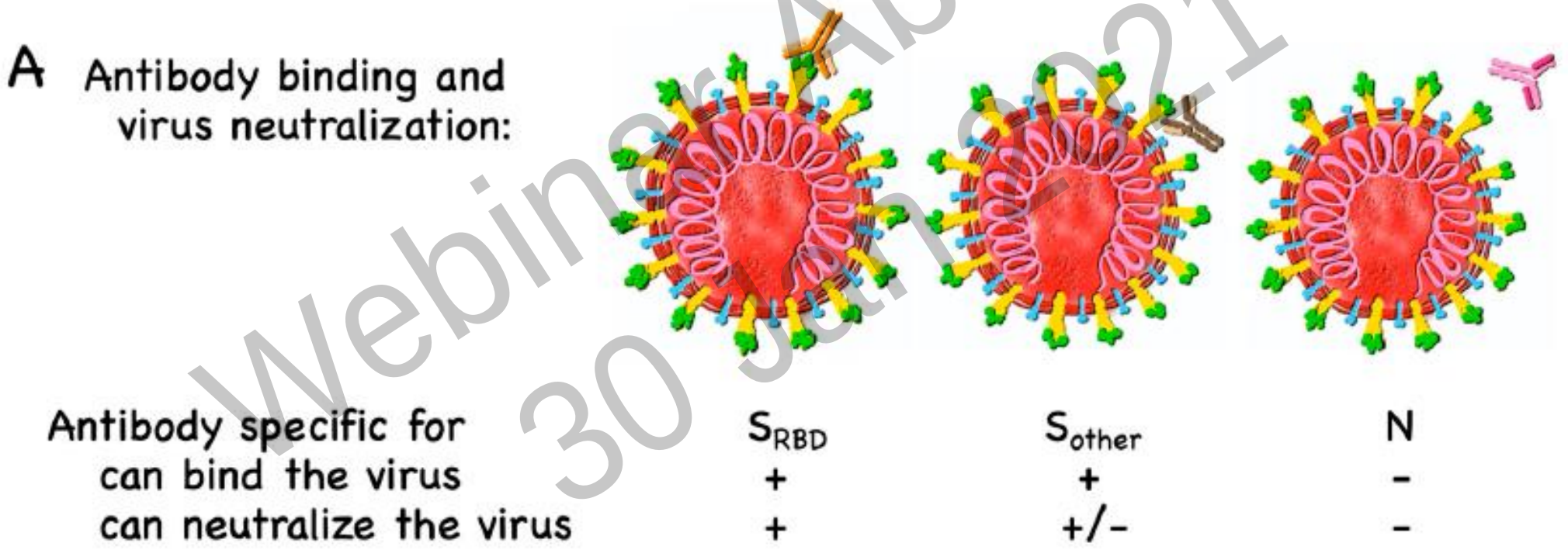
Netralizing Antibdy



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

Different types of antibodies and induction of antibodies by infection and vaccination.

Daniel E. Speiser and Martin F. Bachmann. Vaccines 2020, 8, 404



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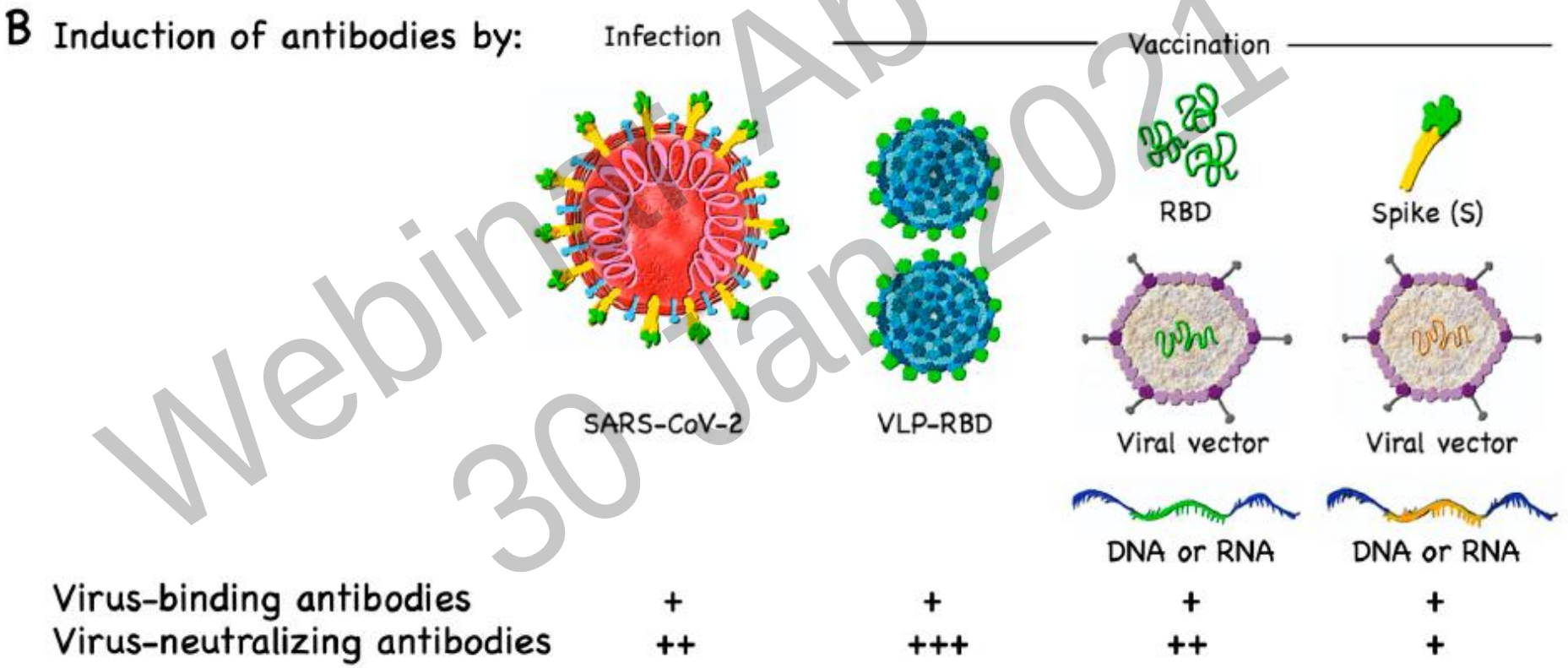


Antibody (Ab) Types & Relevance[1]

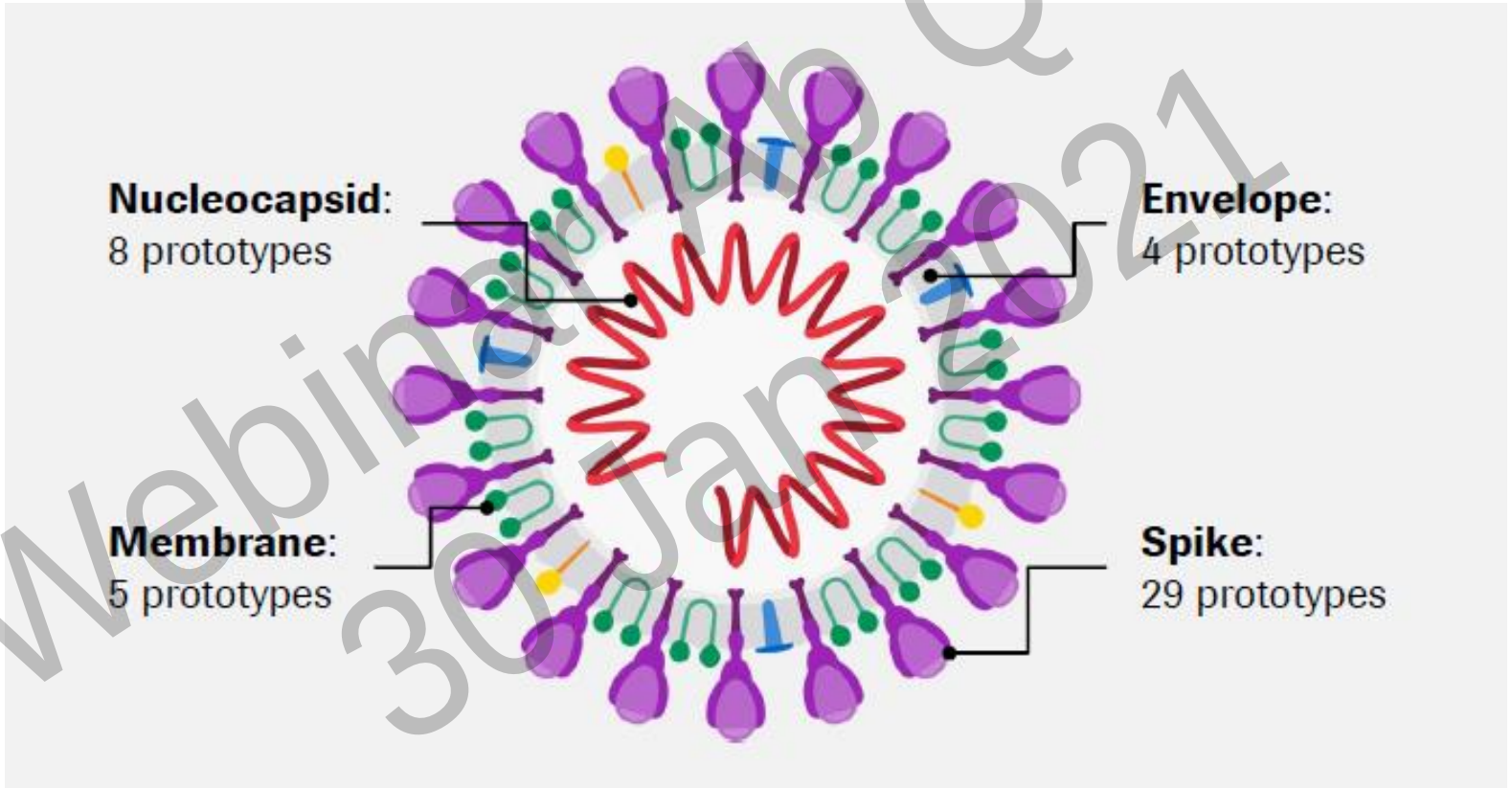
Ab Main Types →	Early/Immature	Mature	Neutralizing
Description	• Appear in early infection phase • **Do not effectively recognize the virus**	• Appear in convalescent phase • **Effectively recognize the virus**	• Appear in convalescent/ immunity phase • **Effectively neutralize the virus**
Examples	IgM, early/immature IgA, IgG	Late/mature Igs	Neutralizing antibodies (*sub-set of mature Igs*)
Relevance/ Purpose	Initial host response to start understanding the virus	Host memory of the virus for future recognition	Render the virus ineffective against the host

All neutralizing Abs are mature Abs BUT not all mature Abs are neutralizing Abs[2]

1. George AJT (2000) The Antibody Molecule. In: George A.J.T., Urch C.E. (eds) Diagnostic and Therapeutic Antibodies. Methods in Molecular Medicine, vol 40. Humana Press;; https://www.britannica.com/science/antibody (accessed April 2020); 2 Roche internal data

R&D approach

- **Parallel testing of 4 different principle antigen designs**
- **4 different target antigens in a total of 46 different configurations**



Elecsys® Anti-SARS-CoV-2

Method for Detection of Neutralizing Antibodies - VNT

Virus Neutralization Test (VNT)

- **"Standard Method"**

VNT is the "Standard method" to evaluate the neutralizing antibodies produced by vaccines.

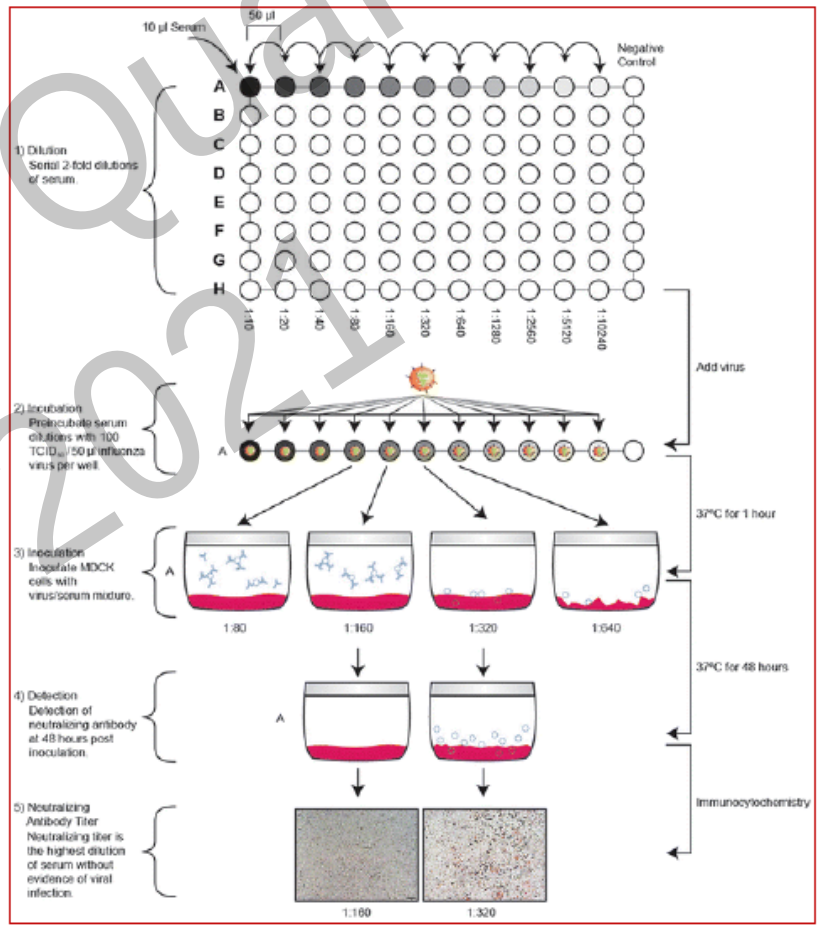
- **High Biosafety requirements**

VNT requires handling **live** SARS-CoV-2 in biosafety laboratory level 3 (BSL3).

- **Time consuming**

VNT needs to culture cells which typically takes **4 days** to complete.

Due to these drawbacks of VNT, immunoassays are most suitable for vaccine evaluation in both **clinical trails** and **national immunization**.



Gauger P.C., Vincent A.L. (2014) Serum Virus Neutralization Assay for Detection and Quantitation of Serum-Neutralizing Antibodies to Influenza A Virus in Swine. In: Spackman E. (eds) Animal Influenza Virus. Methods in Molecular Biology (Methods and Protocols), vol 1161. Humana Press, New York, NY.

J Exp Med. 2020;217(11). doi:10.1084/jem.20201181

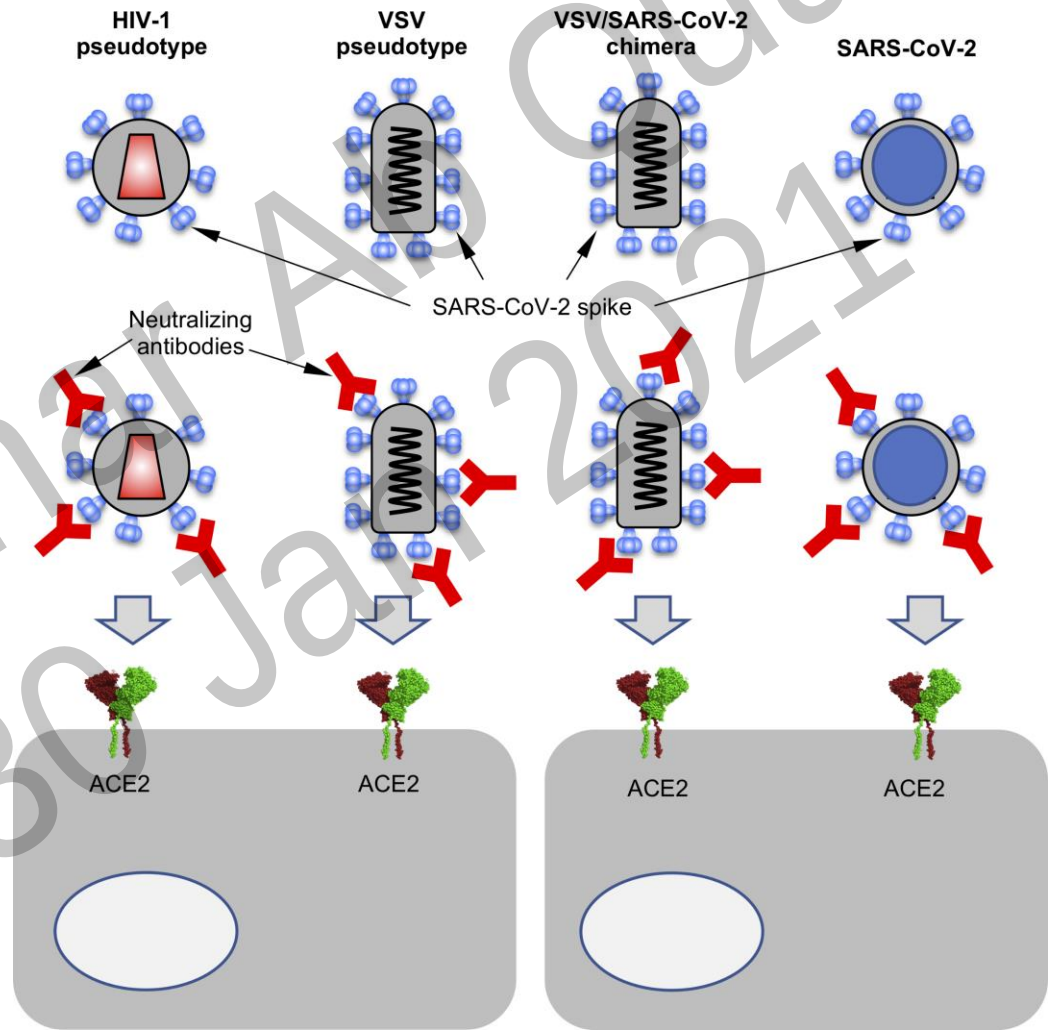
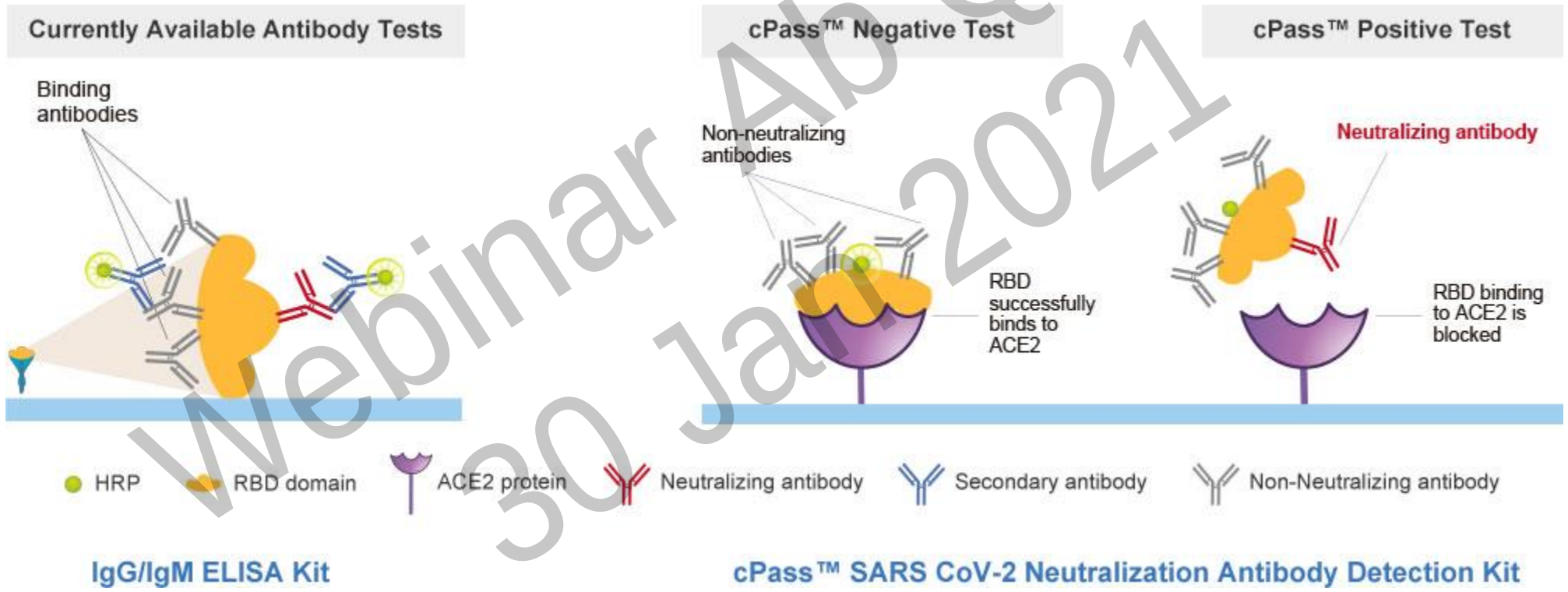


Figure Legend:

Neutralizing Ab Assay

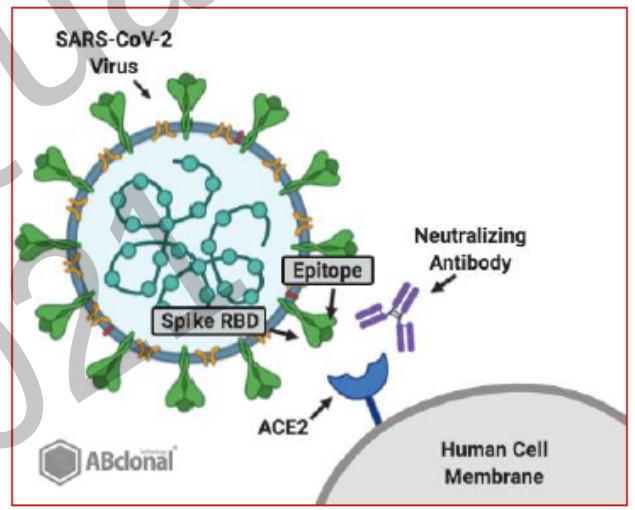


https://www.genscript.com/covid-19-detection-cpass.html

Method for Detection of Neutralizing Antibodies - CLIA

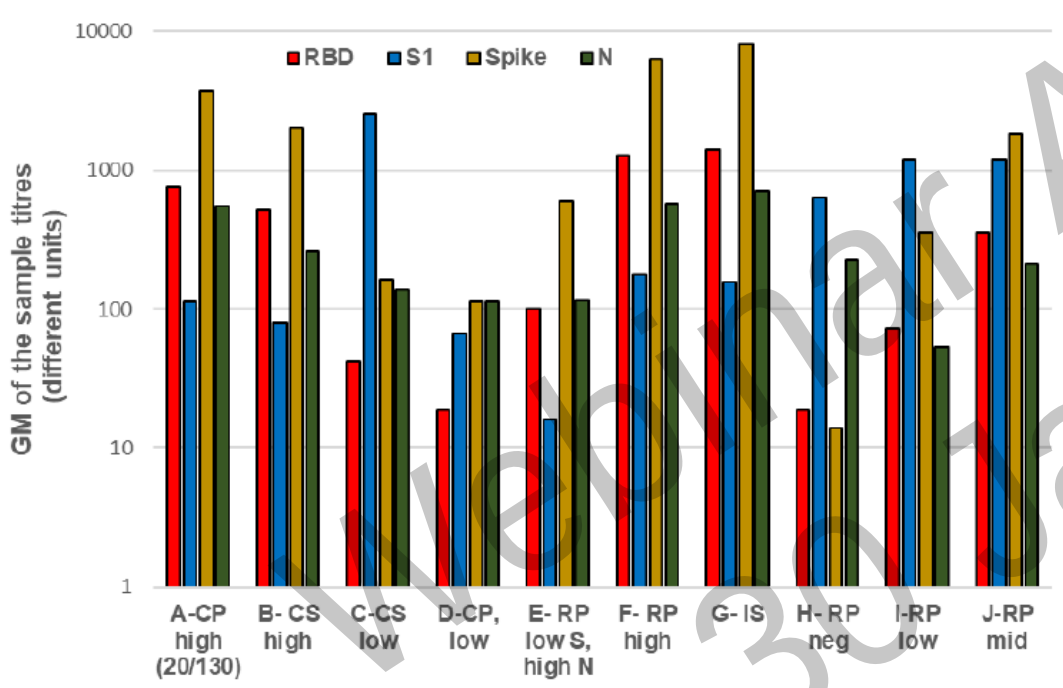
- ✓ Severe Acute Respiratory Syndrome Coronavirus 2 **Neutralizing Antibody** (CLIA)
- ✓ Severe Acute Respiratory Syndrome Coronavirus 2 **S-RBD IgG** (CLIA)

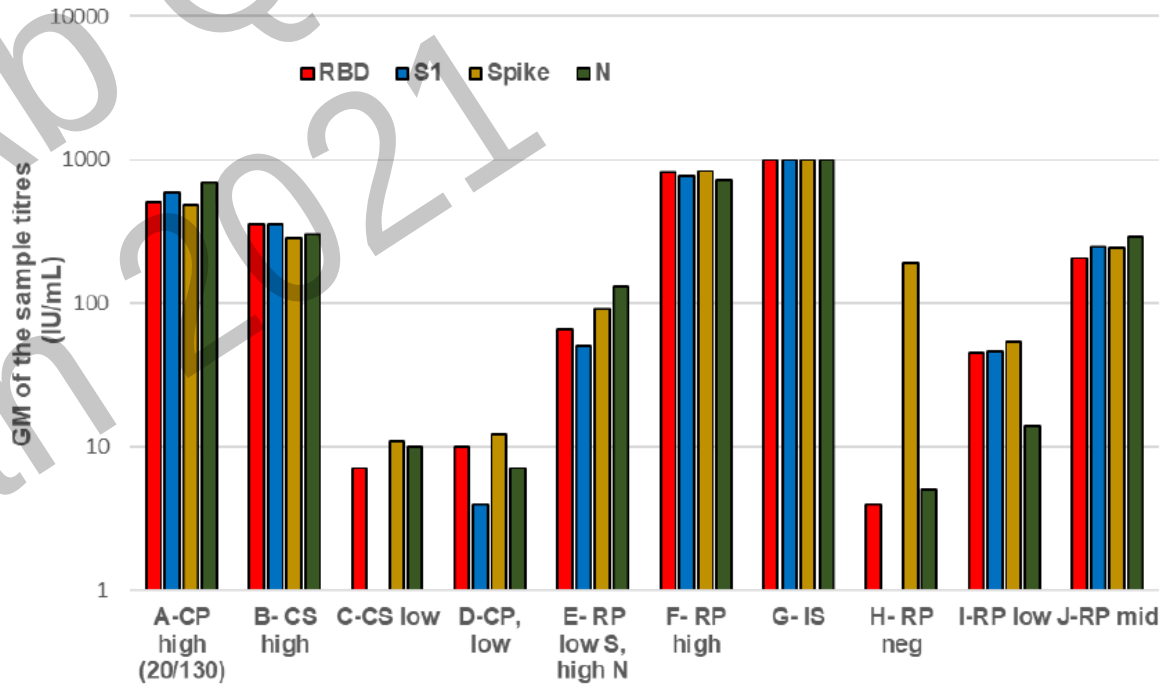
- ➢ **Serological test to detect neutralizing**
- ➢ **Safer :** does not require live pathogen
- ➢ **Quicker:** No need for cell culture, based on a fully-automatic chemiluminescence immunoassay analyzer, assay is suitable for scale up testing



- The design simulates the neutralizing antibodies protection mechanism.
- In this assay, an ACE2 component is introduced to enhance the specificity.
- The binding antibodies without neutral effect cannot be detected.

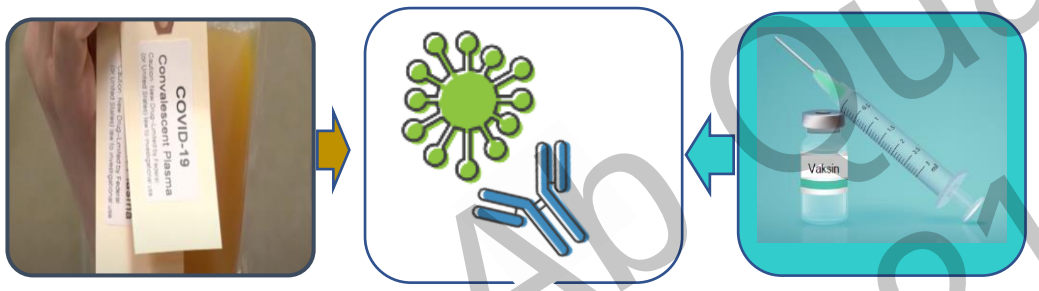
Geometric mean of binding antibody titres for each SARS-CoV-2 antigen





Mattiuzzo G et al. WHO/BS/2020.2403

Research in Progress: ANTIBODI TERHADAP COVID-19



Plasma Antibodi Vaksin

Efektifitas ditentukan oleh **adanya** dan **kadar** antibodi spesifik terhadap COVID-19

1. Mengembangkan *Plaque reduction neutralization test* (PRNT) sebagai "Gold Standard" untuk mengukur kadar antibodi dalam menetralisasi virus
2. Mengembangkan tes imunologi sebagai pengganti PRNT untuk mengukur kadar antibodi spesifik dalam menetralisasi virus
3. Validasi reagen pengganti untuk mengukur kadar antibodi spesifik dalam menetralisir virus

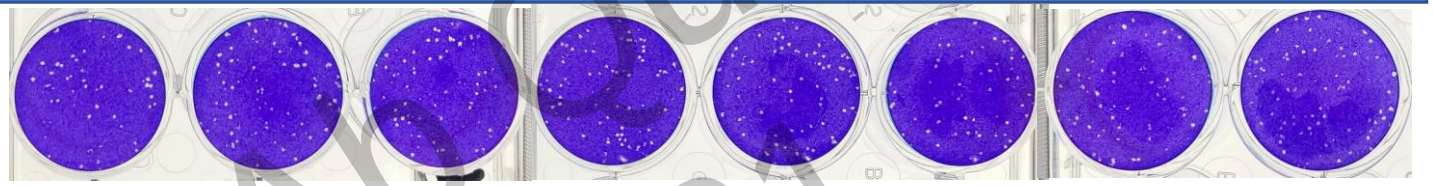
Metode cepat, aman, sederhana, jumlah banyak

Uji $PRNT_{50}$ (*Plaque Reduction Neutralization Test*)

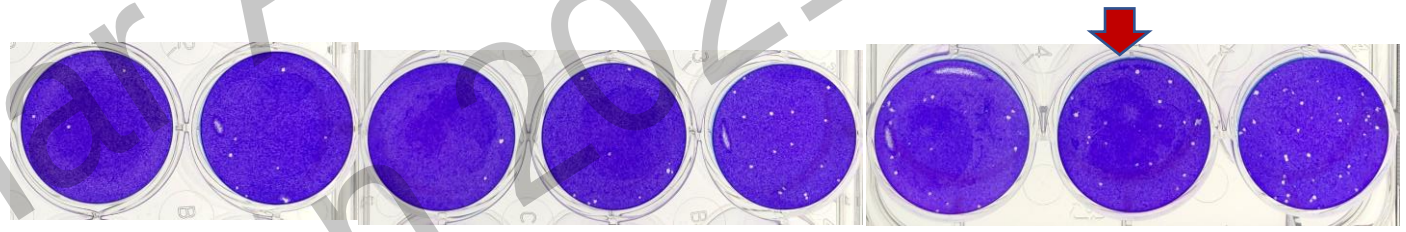
Metode pengukuran *reciprocal* titer antibodi yang dapat menetralisasi virus SARS-CoV-2 dan mereduksi *plaque* hingga 50% dibandingkan dengan kontrol negatif

Pengenceran plasma:	10	20	40	80	160	320	640	1280

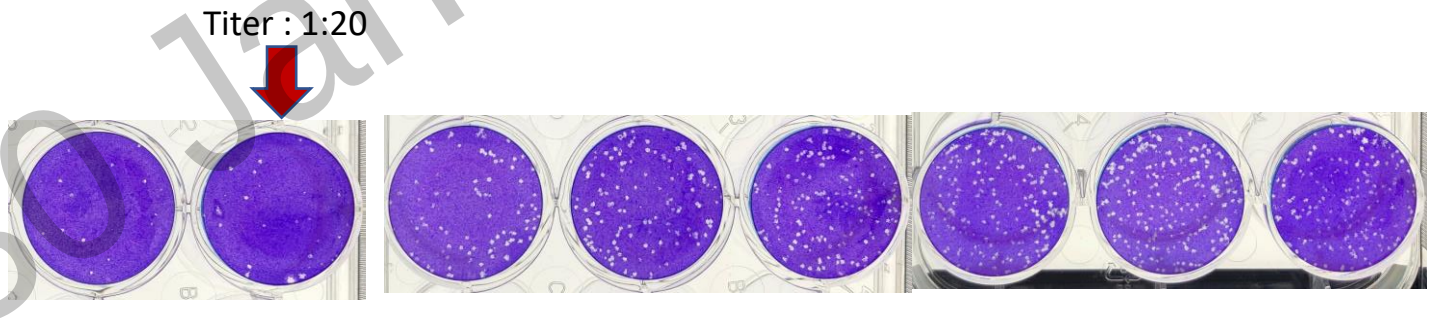
Plasma sehat
(belum memiliki antibodi)



Plasma Penyintas COVID-19 berat
(Kadar antibodi spesifik **tinggi**)
→ Titer PRNT 1:640

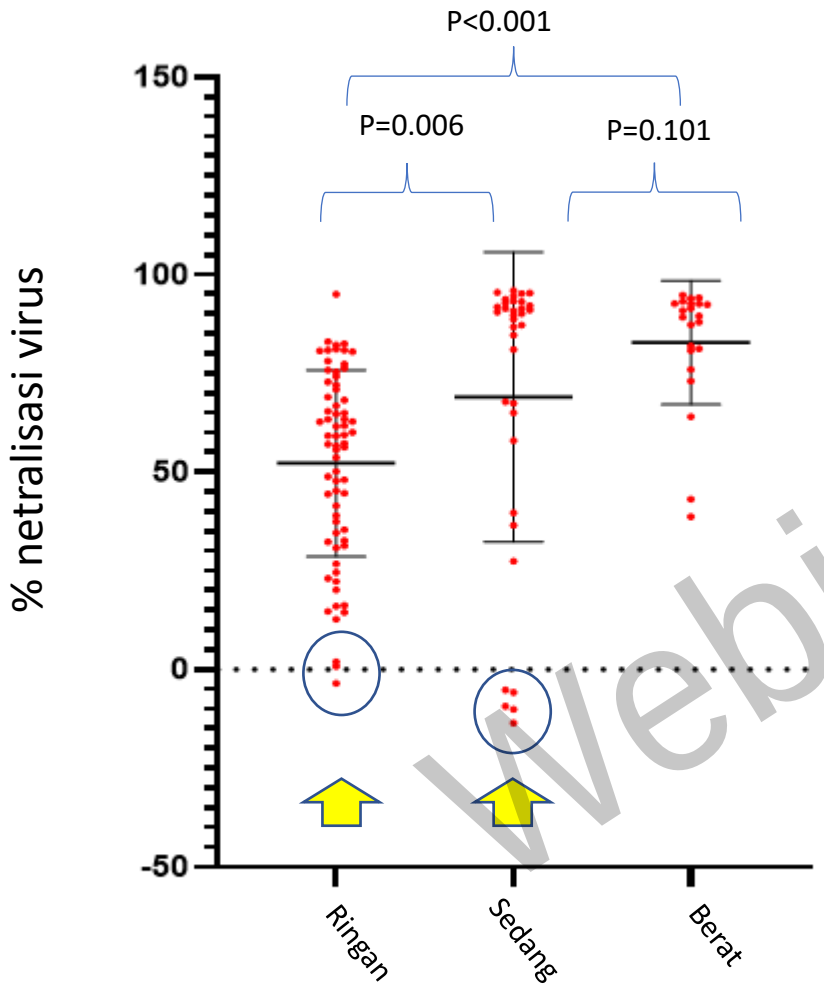


Plasma Penyintas Covid-19 ringan
(Kadar antibodi spesifik **rendah**)
→ Titer PRNT 1:20



- Metode PRNT adalah **baku emas (*gold standard*) untuk uji netralisasi virus**
- Metode PRNT digunakan untuk **mendeteksi dan mengukur kadar antibodi COVID-19** pada Plasma Konvalesen (PK) dan Pasien Penerima Plasma Konvalesens

Kemampuan netralisasi virus antibodi SARS-CoV-2 pada Penyintas COVID-19 berdasarkan derajat penyakit dengan *surrogate method (*metode pengganti) $PRNT_{50}$



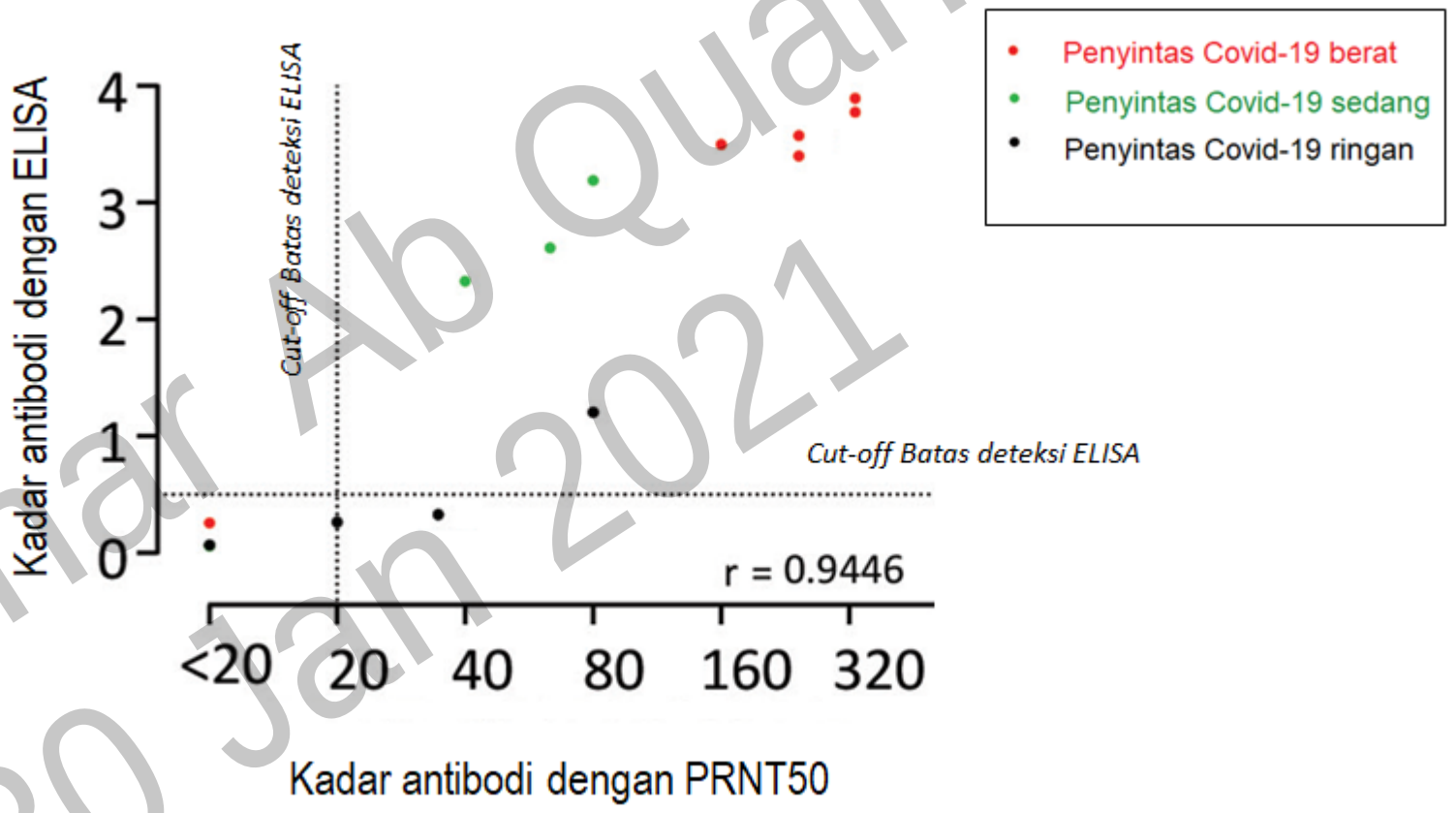
DERAJAT PENYAKIT PENYINTAS COVID-19 SAAT SAKIT

- **Ringan:**
  - kasus ringan (OTG)
  - Isolasi mandiri
- **Sedang:**
  - Kasus sedang
  - Perawatan di RS:: 7-10 hari
  - Tanpa ventilator
- **Berat:**
  - Kasus berat
  - Memakai ventilator
  - Perawatan di RS: >20 hari

- Antibodi COVID-19 pada Penyintas yang menjadi donor Plama Konvalesens (PK): **Lebih tinggi pada Penyintas COVID-19 derajat sedang dan berat** dibanding penyintas derajat ringan
- **Efektivitas Plasma Konvalesen lebih baik pada penderita COVID-19 derajat sedang** dibanding penderita COVID-19 derajat berat/kritis
- **Beberapa pasien sudah memiliki antibodi titer tinggi sebelum mendapat terapi PK** (sudah membentuk antibodi endogen, terutama yang sudah dirawat dalam jangka waktu lama)

Pengembangan Elisa Berdasarkan $PRNT_{50}$ untuk Mengukur Kadar Antibodi Spesifik COVID-19

Pengembangan Bersama Bio Farma Metode Pengukuran Kadar Antibodi dengan ELISA dibandingkan dengan $PRNT_{50}$



- Pengembangan tes imunologi untuk deteksi dan mengukur kadar antibodi virus penyebab COVID-19, dengan PRNT sebagai referensi
- Hasil pengujian selaras dengan Metode $PRNT_{50}$: potensi penggunaan uji ELISA *in-house* deteksi antibodi spesifik RDB SARS-CoV-2
- Manfaat: pengerjaan lebih cepat, dapat dilakukan dalam jumlah banyak, dengan peralatan lebih sederhana, dan tidak memerlukan fasilitas BSL-3

CP Preliminary Study

- Tripartite Collaboration between Eijkman Institute, National Referal Army Hospital, and Bio Farma company.
- First COVID-19 recovered patients has been recruited.
- Ten COVID-19 Patients has been treated
  - 8 Patients with good clinical outcome.
  - 2 end-state patients died.

Consortium for CP Treatment study

Donor: Recovered Covid-19 Patients

- Males preferred.
- Female: No history of pregnancy
- Healthy (Physically, confirmed by Lab Tests)
- Free of CoV and *Transfusion Transmitted Infection*
- Enough titer of anti-Covid-19 antibody (PRNT)

Plasma (by Certified BTU)

- Plasmapheresis
- Storage
- Delivery

Patients

- Indication
- Doses
- Monitoring and Evaluation
- Reporting

Ehtical Clearance	
Development of National Protocol	
MoH	• Hospital
Ina FDA	• Investigational New Drug
Red-cross	• CP Processing
Eijkman Inst.	• PRNT (BSL3)

Consortium for CP Treatment study: Progress

- Ina-FDA Guidelines has been issued.
- National Protocol for CP treatment of COVID-19 Patients has been developed.
- COVID-19 PRNT has been developed by Eijkman Institute
- This Clinical Trial has been Registered at ClinicalTrials.Gov PRS (*Protocol Registration and Results System*) – Protocol ID No: 3471041S322342020040800002

Under-construction

- **Kesesuaian Uji Pengukuran nAB Komersial vs. PRNT:**
  - **Kualitatif?**
  - **Kuantitatif?**

Thank you