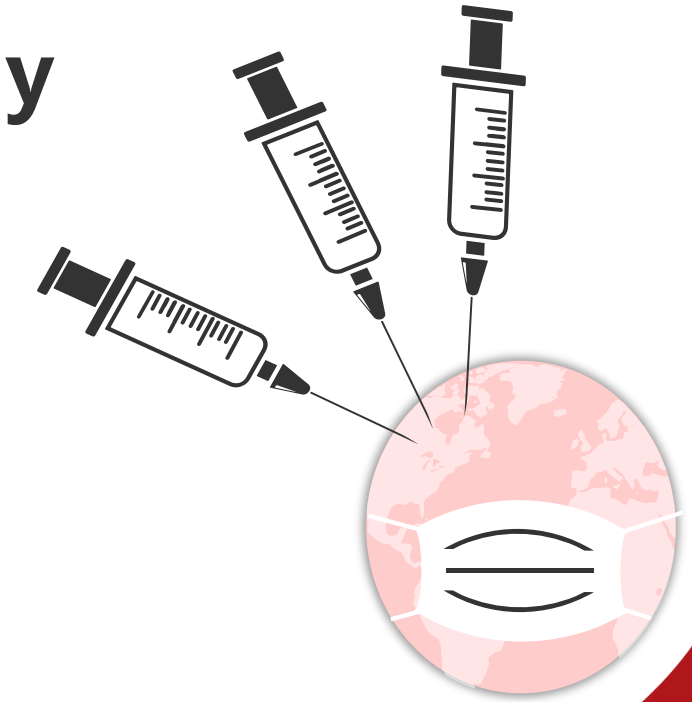
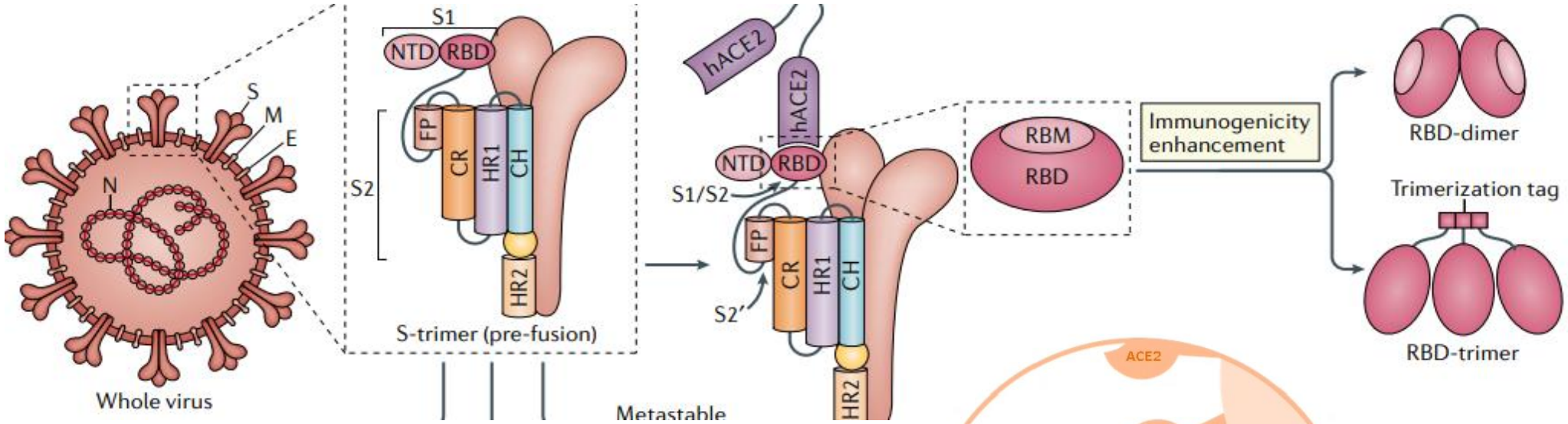
Mindray Neutralizing Antibody Assays Information



Neutralizing antibodies and Vaccines

Neutralizing antibodies bind to the protein, and prevent recognition of the ACE2 receptor and or viral fusion



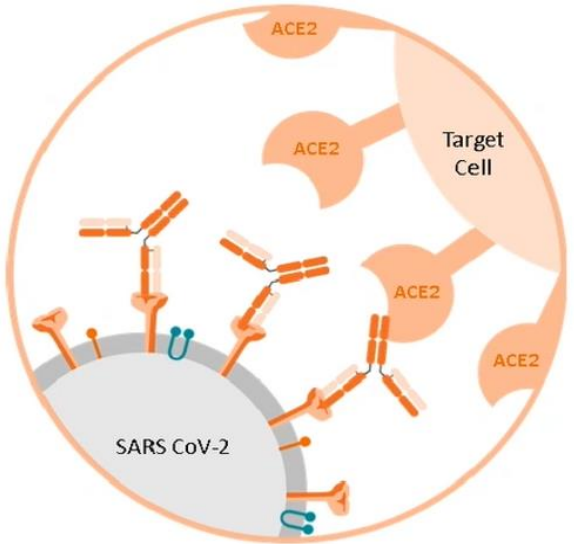
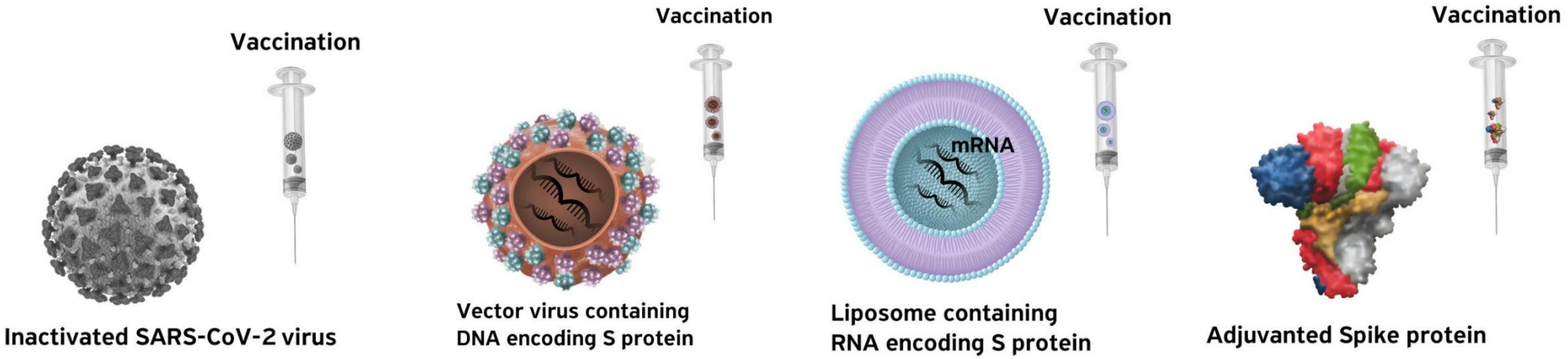


Image from:
https://www.prosci-inc.com/ace2-antibodies/

SARS-CoV-2 Neutralizing Antibody Mechanism

Leading vaccine candidates against SARS-COV-2



COVID-19 vaccines using this platform	Sinopharm, Sinovac, Bharat Biotech and etc.	CanSino, Oxford-AstraZeneca, Gamaleya, Johnson & Johnson	Pfizer-BioNTech, Moderna and etc.	Vector Institute, Novavax, Anhui Zhifei Longcom and etc.
Phase and status	● **Sinopharm-Phase 3**-Approved in China, U.A.E., Bahrain. Emergency use in other coutries. ● **Sinovac-Phase 3**-Approved in China. Emergency use in other countries. ● **Bharat Biotech-Phase 3**-Emergency use in India, Iran, Zimbabwe.	● **CanSino-Phase 3**-Approved in China. Emergency use in Mexico, Pakistan. ● **AstraZeneca-Phase 2/3**-Approved in Brazil. Emergency use in U.K., E.U., other countries. ● **Gamaleya-Phase 3**-Early use in Russia. Emergency use in other countries. ● **J&J-Phase 3**-Emergency use in U.S., E.U., Bahrain.	● **Pfizer-BioNTech-Phase 2/3**-Approved in several countries. Emergency use in U.S., E.U., other countries. ● **Moderna-Phase 3**-Approved in Switzerland. Emergency use in U.S., U.K., E.U., others.	● **Vector Institute-Phase 3**-Early use in Russia. ● **Novavax-Phase 3**. ● **Anhui Zhifei Longcom-Chinese Academy of Medical Sciences-Phase 3**-Emergency use in China and Uzbekistan.
Mindray' solution for monitoring vaccine-induced immune response	***Anti-S-RBD IgG/Total antibody***	***Neutralizing Antibody/Anti-S-RBD IgG/Total antibody??***	***Neutralizing Antibody/Anti-S-RBD IgG/Total antibody??***	***Anti-S-RBD IgG/Total antibody??***

Image from: Pediatric Allergy and Immunology, Volume: 32, Issue: 1, Pages: 9-16, First published: 28 October 2020, DOI: (10.1111/pai.13401)
Data from: https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html

How the inactivated vaccine Works

Vaccine developer	Number of doses	Dosing schedule(day)	Trial group	Monitoring time of neutralizing antibody(day)	Efficacy
Sinopharm	2	0+21 (Phase 2)	1. 18–59 years	PRNT:0+35+49 (peak-28 days after the second inoculation) [1] Neutralizing antibody GMT: 282.7149 specific IgG-binding antibody:0+35 (peak-14 days after the second inoculation) [2] IgG titers (to whole SARS-CoV-2 antigen): 215	79.34%
		0+28 (Phase 1)	2. ≥60 years	PRNT:0+7+14+28+32+42 (peak-14 days after the second inoculation) [1] Neutralizing antibody GMT: 131.4649; Seroconversion rate: 100%	
Sinovac Biotech	2	0+14 (Phase 2)	1. 18–59 years	PRNT:0+28+42 (peak-14 days after the second inoculation) [3] Neutralizing antibody GMT: 27.6; Seroconversion rate: 92.4% RBD specific IgG:0+28+42 (peak-14 days after the second inoculation) IgG titers: 1094.3; Seroconversion rate: 96.5%	50.38%-Brazil trial 83.5%-Turkey trial
		0+28 (Phase 1)	2. ≥60 years	PRNT:0+28+56 (peak-28 days after the second inoculation) [4] Neutralizing antibody GMT: 54.9; Seroconversion rate: 100.0%	

How the mRNA vaccine Works

Vaccine developer	Number of doses	Dosing schedule(day)	Trial group	Monitoring time of neutralizing antibody(day)	Human convalescent serum	Efficacy
Moderna	2	0+28	1. 18-55 years	Live virus $PRNT_{80}$:0+42 (peak-14 days after the second inoculation)[5] GMR: 654.3 IgG ELISA-S-2P:0+14+28+35+42+56 (peak-14 days after the second inoculation) GMT: 811,119 IgG ELISA-RBD:0+14+28+35+42+56 (peak-14 days after the second inoculation) GMT: 558,905	Live virus $PRNT_{80}$:158.3 IgG ELISA-S-2P:142,140 IgG ELISA-RBD:37,857	94.10%
			2. 56-70 years	Live virus $PRNT_{80}$:0+42 (peak-14 days after the second inoculation)[6] GMR: 878 IgG ELISA-S-2P:0+14+28+35+42+56 (peak-7 days after the second inoculation) GMT: 5,033,017 IgG ELISA-RBD:0+14+28+35+42+56 (peak-7 days after the second inoculation) GMT: 1,471,882	Live virus $PRNT_{80}$:158 IgG ELISA-S-2P:138,901 IgG ELISA-RBD:37,244	
			3. ≥71 years	Live virus $PRNT_{80}$:0+42 (peak-14 days after the second inoculation)[6] GMR: 317 IgG ELISA-S-2P:0+14+28+35+42+56 (peak-7 days after the second inoculation) GMT: 2,636,979 IgG ELISA-RBD:0+14+28+35+42+56 (peak-7days after the second inoculation) GMT: 711,752		
Pfizer-BioNTech	2	0+21	1. 18-55 years	PRNT:0+7+21+28+42+49+84 (peak-7days after the second inoculation)[7] Neutralization Titer:312 S1 specific IgG:0+7+21+28+42+49+84 (peak-7days after the second inoculation) (U/ml): 8,279 RBD specific IgG:0+7+21+28+42+49+84 (peak-7days after the second inoculation) (U/ml): 6,299	PRNT50-Neutralization Titer:94 S1-IgG (U/ml): 631 RBD-IgG (U/ml):602	91.30%
			2. 65-85 years	$PRNT_{50}$:0+21+28+35 (peak-14 days after the second inoculation)[8] Neutralization Titer: 206 S1-Binding IgG:0+21+28+35 (peak-7days after the second inoculation) (U/ml): 7,985	PRNT50-Neutralization Titer:94 S1-Binding IgG-(U/ml): 631	

How the Viral vector-based vaccine Works

Vaccine developer	Number of doses	Dosing schedule(day)	Trial group	Monitoring time of neutralizing antibody(day)	Human convalescent serum	Efficacy
Gamaleya	2	0+21	18–60 years	PRNT:0+14+28+42 (peak-21days after the second inoculation)[9] GMT: 49.25; Seroconversion rate:100% RBD specific IgG:0+14+21+28+42 (peak-21days after the second inoculation) GMT: 14703; Seroconversion rate:100%	at ~1 month after recovery PRNT- GMT: 32.96; Seroconversion rate:90.9% RBD IgG- GMT: 1266; Seroconversion rate:85.8%	91.60%
AstraZeneca	2	0+28	1. 18-55 years	live SARS-CoV-2 micro-neutralization assay MNA_{80}:0+28+42 Median: 136[10] Standardized IgG against trimeric SARS-CoV-2 spike protein: 0+7+14+28+35+42+56 (peak-14 days after the second inoculation) EU: 997.5 Multiplex MSD – Anti spike IgG : 0+28+42 (peak-14 days after the second inoculation)-(AU/mL): 33830.8 Multiplex MSD – RBD IgG: 0+28+42 (peak-14 days after the second inoculation)-(AU/mL): 16825.4	/	81·3%
			2. 56-69 years	live SARS-CoV-2 micro-neutralization assay MNA_{80}:0+28+42+56 Median: 144[11] Standardized IgG against trimeric SARS-CoV-2 spike protein: 0+14+28+35+42+56 (peak-14 days after the second inoculation) EU: 648 Multiplex MSD – Anti spike IgG : 0+28+42+56 (peak-14 days after the second inoculation)-(AU/mL): 20617		
			3. ≥70 years	live SARS-CoV-2 micro-neutralization assay MNA_{80}:0+28+42+56 Median: 161[11] Standardized IgG against trimeric SARS-CoV-2 spike protein: 0+14+28+35+42+56 (peak-14 days after the second inoculation) EU: 525 Multiplex MSD – Anti spike IgG : 0+28+42+56 (peak-14 days after the second inoculation)-(AU/mL): 19414		
Johnson & Johnson	1	/	1. 18-55 years	PRNT:0+28+56+70 (peak-70 days after inoculation)[12] (IC50 Log_{10}GMT): 321; Seroconversion rate:100% Anti spike IgG:0+28+56+70 (peak-56 days after inoculation) (EU/ml Log_{10}GMC): 660; Seroconversion rate:100%	PRNT-Log_{10}GMT:522 Anti spike IgG-Log_{10}GMC:899	72%-United States, 64%-South Africa, 61%-Latin America
			2. >65 years	PRNT:0+14+28 (peak-28 days after inoculation) (IC50 Log_{10}GMT): 277; Seroconversion rate:96% Anti spike IgG:0+14+28 (peak-28 days after inoculation) (EU/ml Log_{10}GMC): 312; Seroconversion rate:96%		

How the Subunit vaccine Works

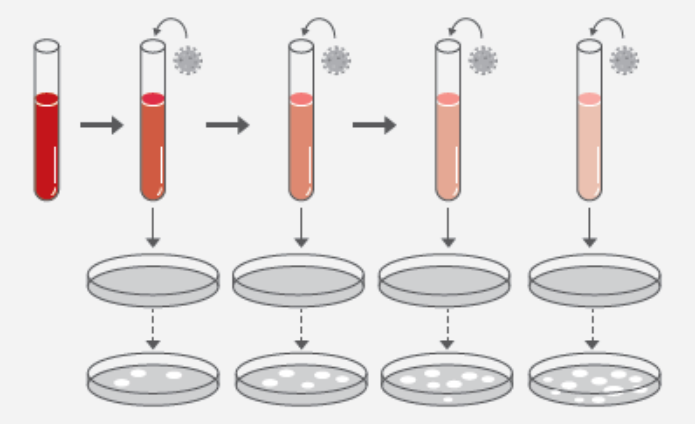
Vaccine developer	Number of doses	Dosing schedule(day)	Trial group	Monitoring time of neutralising antibody(day)	Human convalescent serum	Efficacy
Novavax	2	0+21	18–59 years	PRNT($MN_{IC>99\%}$):0+21+35 (peak-14 days after the second inoculation)[13] GMT:3906 Anti-Spike IgG:0+7+21+28+35 (peak-14 days after the second inoculation) (EU/ml): 63,160	PRNT: 983; S- IgG: 8344 Asymptomatic: P-254; S-1661 Outpatient symptomatic: P-837; S-7420 Hospitalized: P-7457; S-53,391	89.30%
ZFSW	3	0+30+60	18–59 years	$PRNT_{50}$:0+44+74 (peak-14 days after the third inoculation)[14] GMT: 103; Seroconversion rate: 96.6% RBD specific IgG:0+14+30+44+60+74 (peak-14 days after the third inoculation) GMT: 1782; Seroconversion rate: 99.3%	$PRNT_{50}$- GMT: 51	Unknown

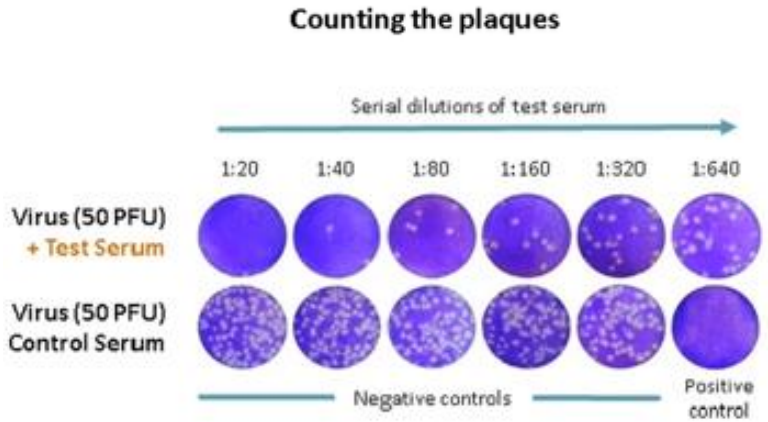
Image from: https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html

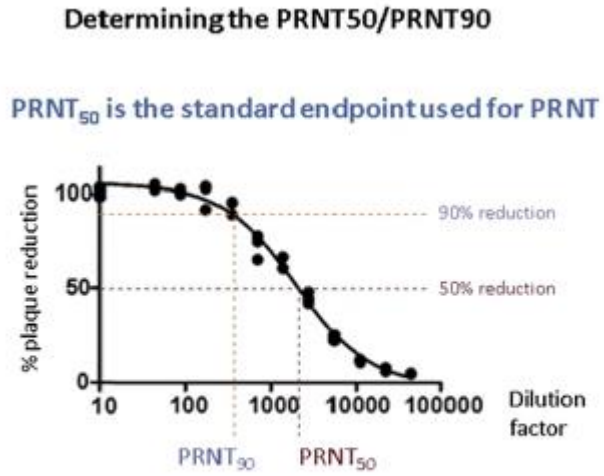
Do we have any effective methods to detect neutralizing antibodies?

Conventional approach- Virus Neutralization Test (VNT)

- **"Standard Method"**
- **High Biosafety requirements**
- **Time consuming**
- **Very limited lab resources and high cost**





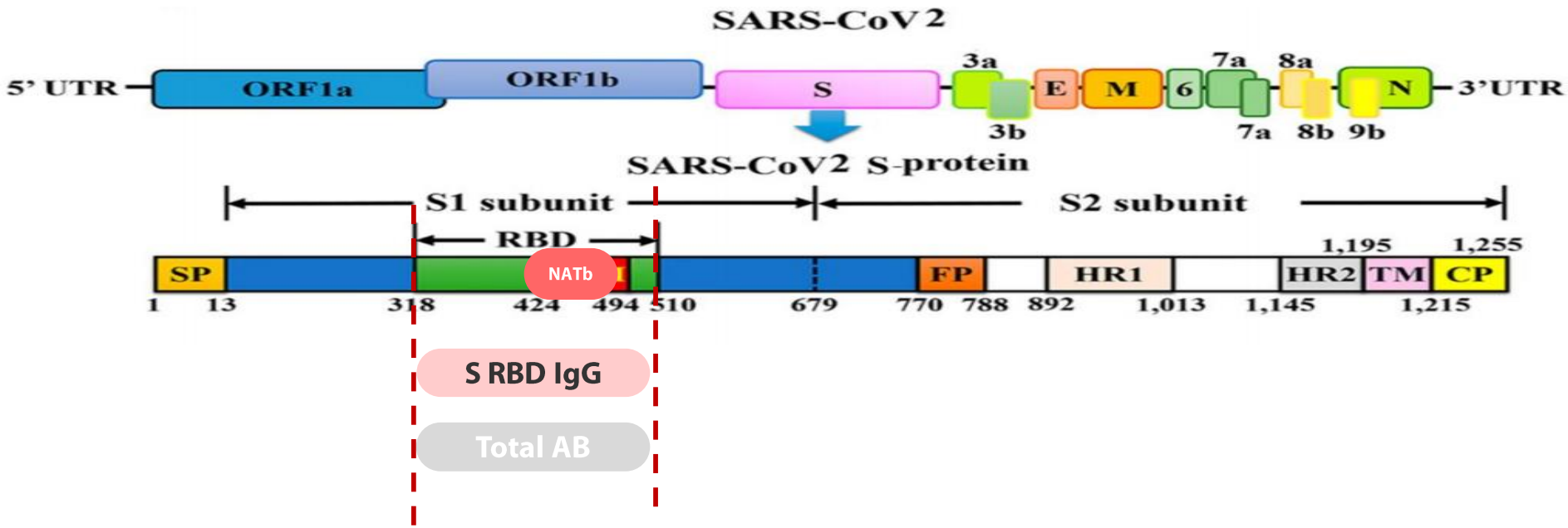


PRNT$_{50}$ is the maximum serum dilution that results in ≥ 50% reduction in plaque count

Because of these drawbacks of VNT, immunoassays are necessary to vaccine evaluation in both clinical trails and national immunization.

Immunoassay for detection of neutralizing antibodies

Neutralizing antibody tests are designed to reflect the protection mechanism in vivo



Neutralizing antibody is targeting special sites of S RBD

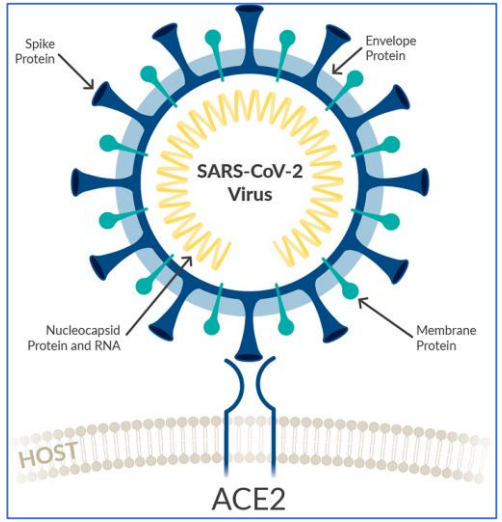
S-RBD IgG is targeting the whole sequence of RBD, BUT only detect IgG type

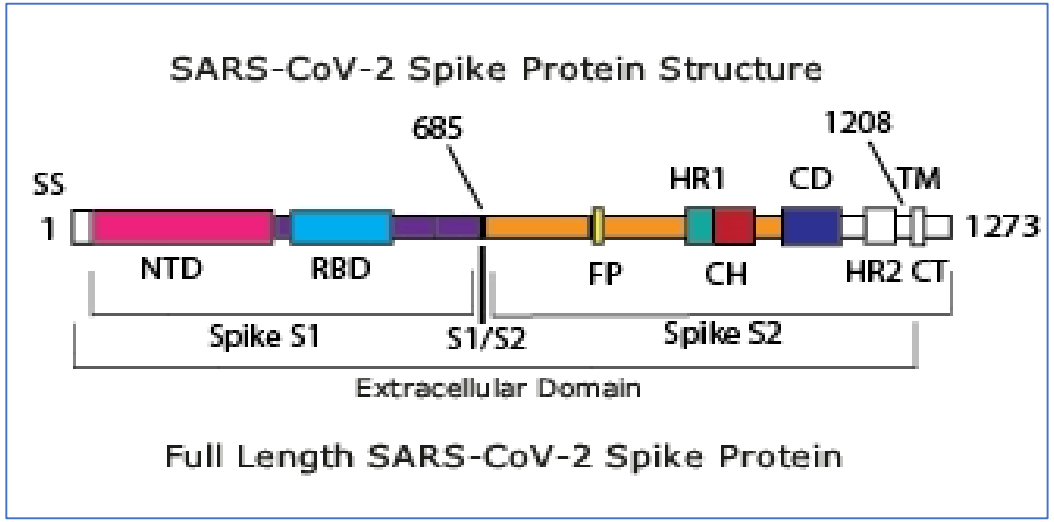
Total antibody is target the whole sequence of RBD, BUT detect IgM, IgG and IgA together.

Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment

What kinds of serology tests can Mindray provide in the post pandemic era?

SARS-COV-2 Antibody Types, Functions and Possible Clinical Scenarios

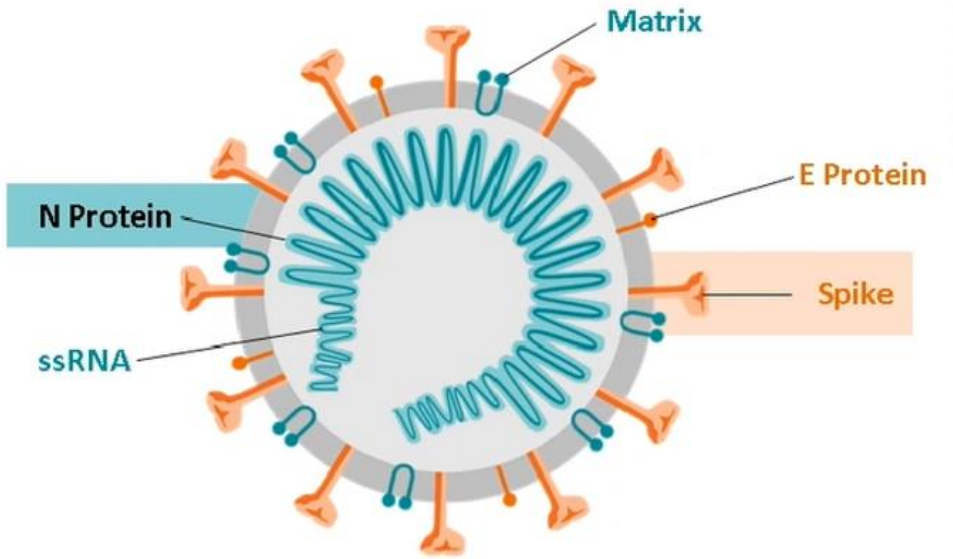


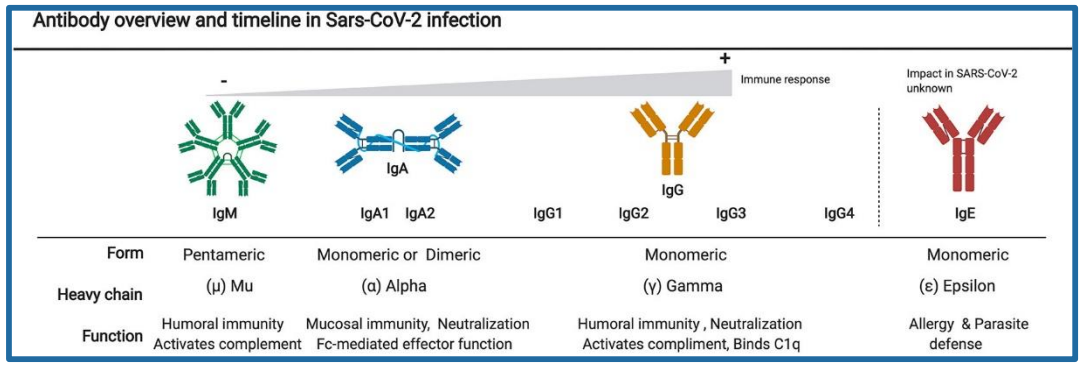


- The S protein binds to ACE2 through the RBD region of the S1 subunit, mediating **viral attachment** to host cells.
- The FP of SARS-CoV-2 and the two HR domains on S2 are essential for **viral fusion** to host cells.

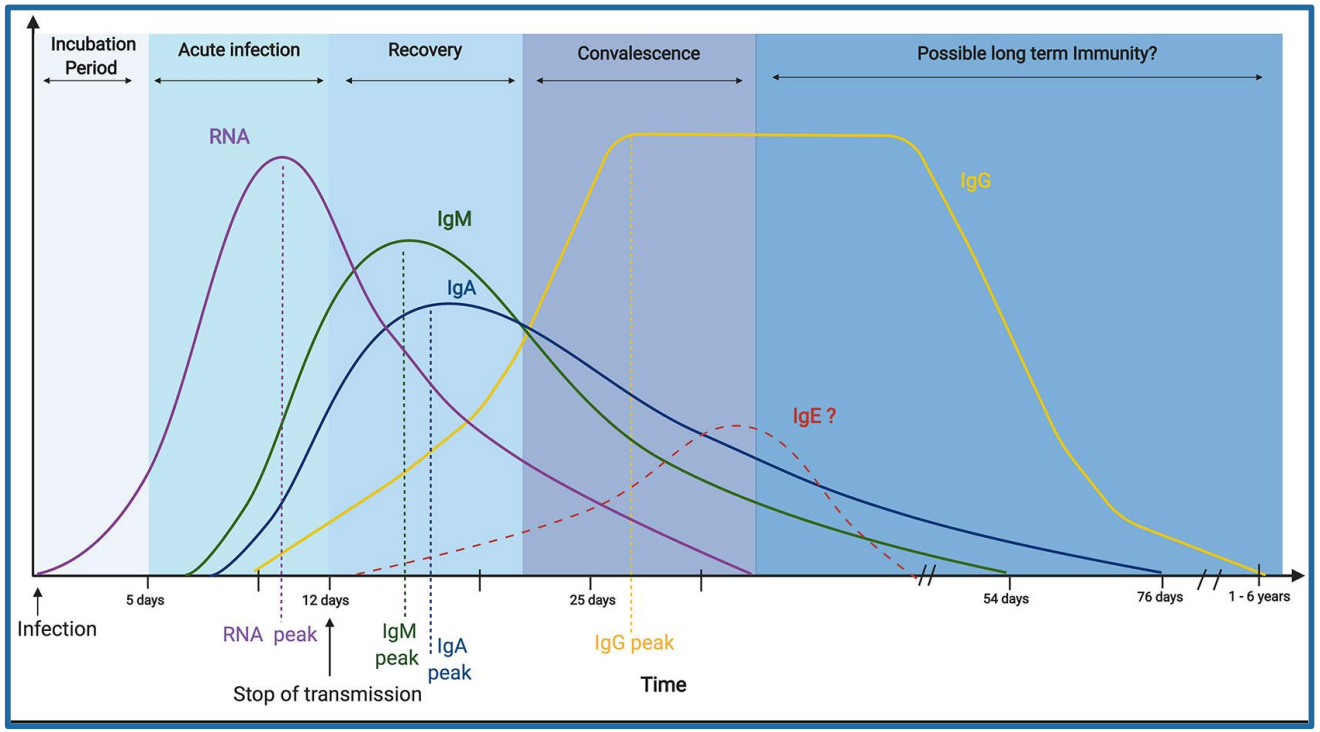
Antibody	Main Function	Possible Clinical Scenario	Competitors with same design	Progress
IgG and IgM	**IgG and IgM** to **RBD on S-protein + N protein**	Aid to diagnosis, indicate disease course		Already launched
Neutralizing Antibody *(Core)*	special sites of S RBD	**May indicate natural infection and vaccine induced immunity**	GenScript	Already launched
Neutralizing Antibody *(S-RBD IgG)*	the whole sequence of RBD, BUT only detect IgG type	Indicate past infection May indicate herd immunity **May indicate natural infection and vaccine induced immunity**	Siemens, Abbott	Already launched
Neutralizing Antibody *(Total antibody)*	the whole sequence of RBD, BUT detect IgM, IgG and IgA together	Aid to diagnosis Prevalence screening **May indicate natural infection and vaccine induced immunity**	Roche	under development

IgM & IgG



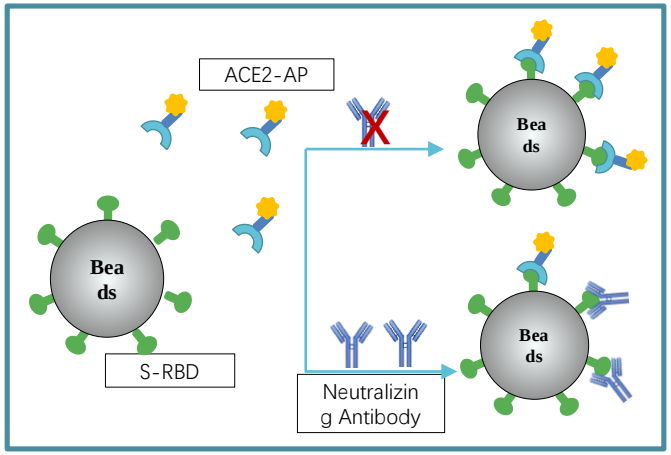


Help doctors evaluate the dynamics of the immune response of patients depending on the duration of the disease!



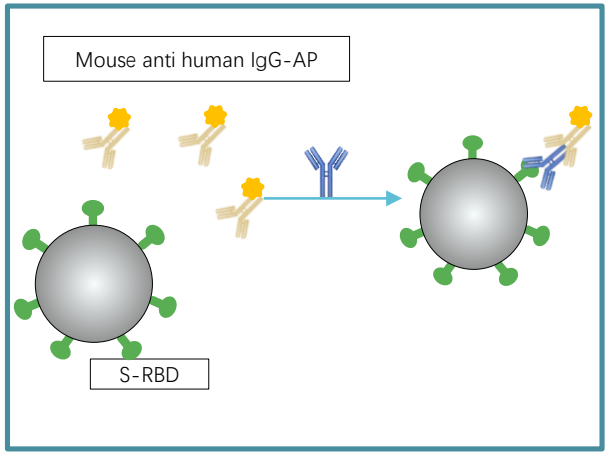
Neutralizing antibody tests

The design of NTAb assay



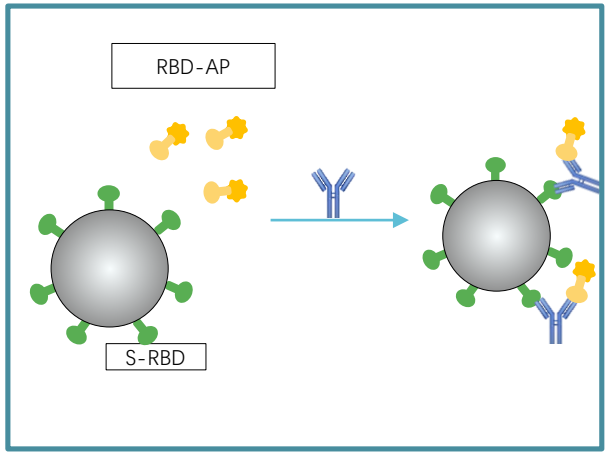
competitive method

The design of RBD IgG assay



Indirect mothed

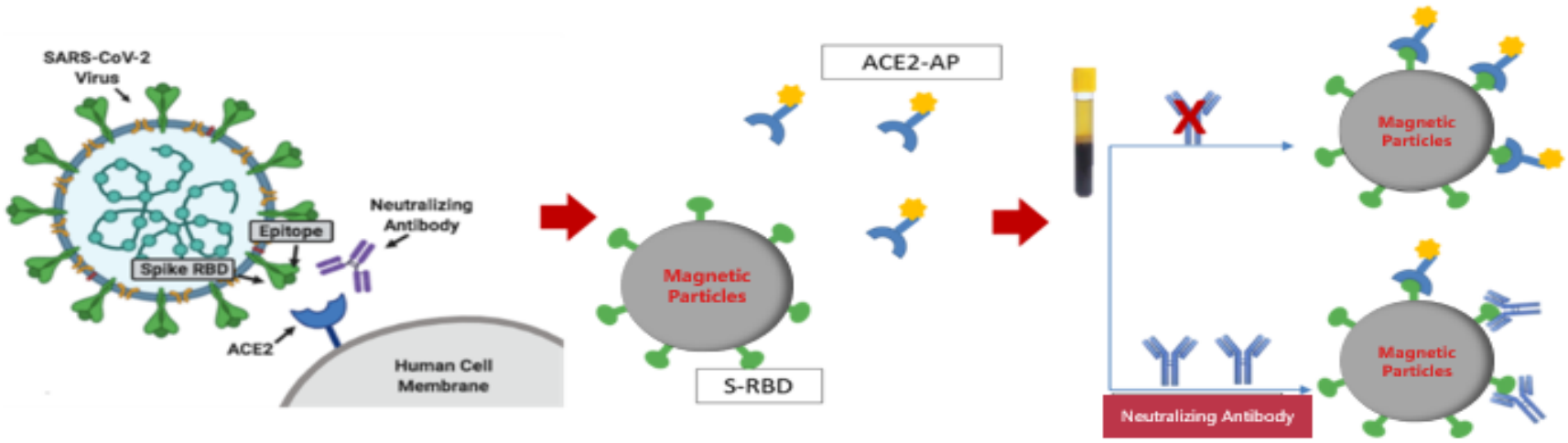
The design of Total antibody assay



Sandwich mothed

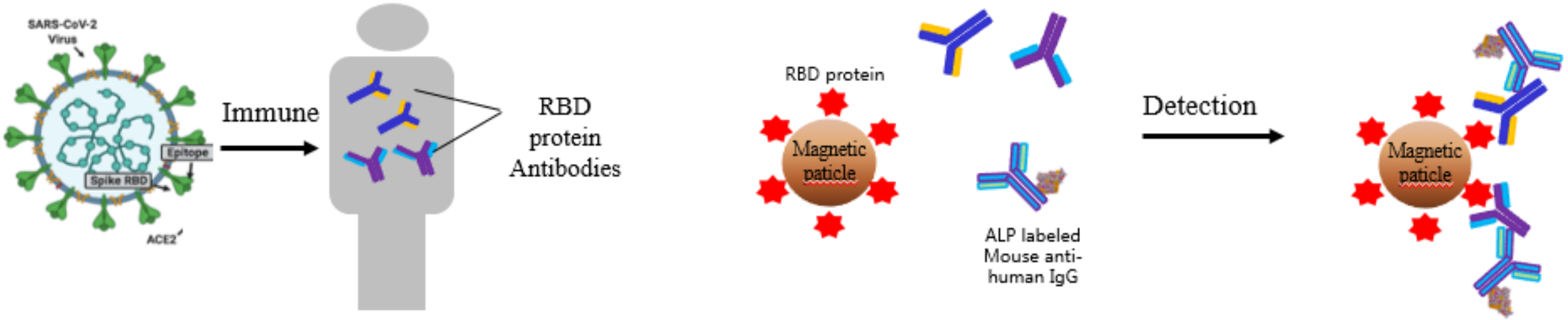
Antibody	Main Function	Possible Clinical Scenario	Competitors with same design	Progress
IgG and IgM	**IgG and IgM** to **RBD on S-protein + N protein**	Aid to diagnosis, indicate disease course	Snibe, YHLO	Already launched
Neutralizing Antibody *(Core)*	special sites of S RBD	**May indicate natural infection and vaccine induced immunity**	GenScript, Snibe	launched
Neutralizing Antibody *(S-RBD IgG)*	the whole sequence of RBD, BUT only detect IgG type	Indicate past infection May indicate herd immunity **May indicate natural infection and vaccine induced immunity**	Snibe, Siemens, Abbott	launched
Neutralizing Antibody *(Total antibody)*	the whole sequence of RBD, BUT detect IgM, IgG and IgA together	Aid to diagnosis Prevalence screening **May indicate natural infection and vaccine induced immunity**	Roche	under development

Test Principle-Neutralizing Antibody



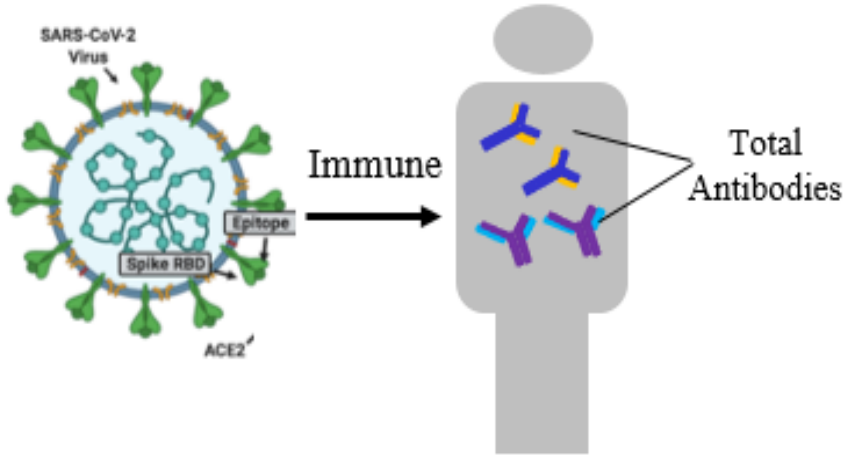
competitive method

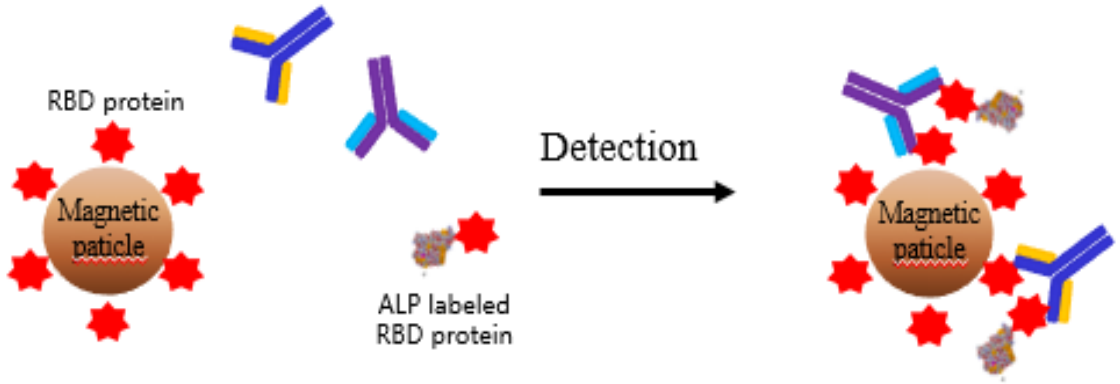
Test Principle-S-RBD IgG Assay



Indirect method

Test Principle-Total Antibody Assay





Sandwich method

Interpretation of Results

1. Non-reactive: S-RBD IgG<10, Total antibody<10, Neutralizing antibody<10
2. Reactive: S-RBD IgG≥10, Total antibody≥10, Neutralizing antibody≥10

As with all analyte determinations, the result should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

The individual immune response following SARS-CoV-2 infection varies considerably and might give different results with assays from different manufacturers. Results of assays from different manufacturers should not be used interchangeably.

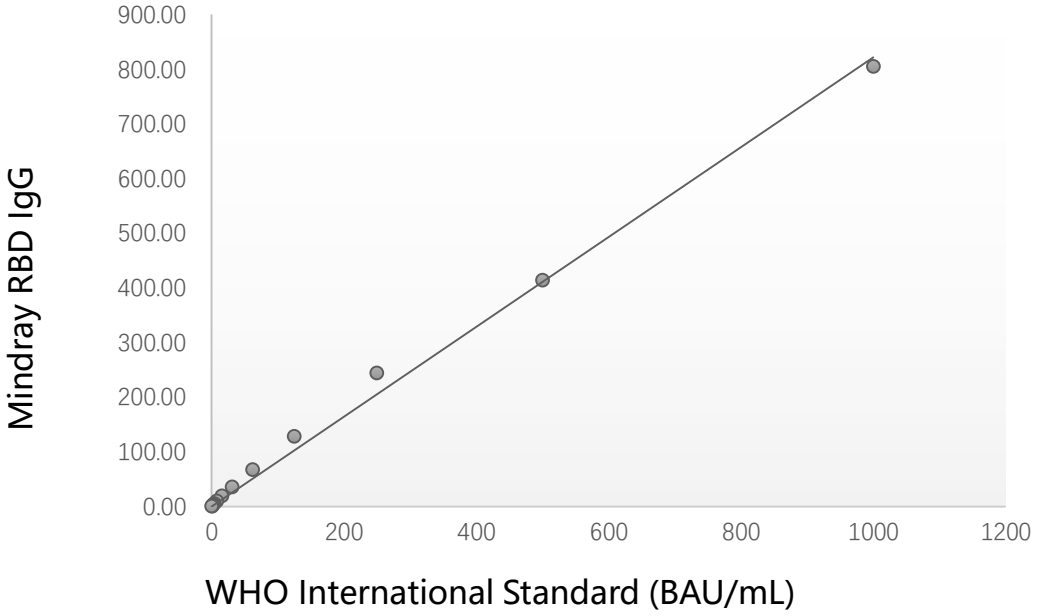
WHO Standardization Results of S RBD IgG

Medicines & Healthcare products Regulatory Agency

WHO International Standard
First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human)
NIBSC code: 20/136
Instructions for use
(Version 2.0, Dated 17/12/2020)

1. INTENDED USE

The First WHO International Standard for anti-SARS-CoV-2 immunoglobulin is the freeze-dried equivalent of 0.25 mL of pooled plasma obtained from eleven individuals recovered from SARS-CoV-2 infection. The preparation has been evaluated in a WHO International Collaborative study (1). The intended use of the International Standard is for the calibration and harmonisation of serological assays detecting anti-SARS-CoV-2 neutralising antibodies. The preparation can also be used as an internal reference reagent for the harmonisation of binding antibody assays. The preparation has been solvent-detergent treated to minimise the risk of the presence of enveloped viruses (2).



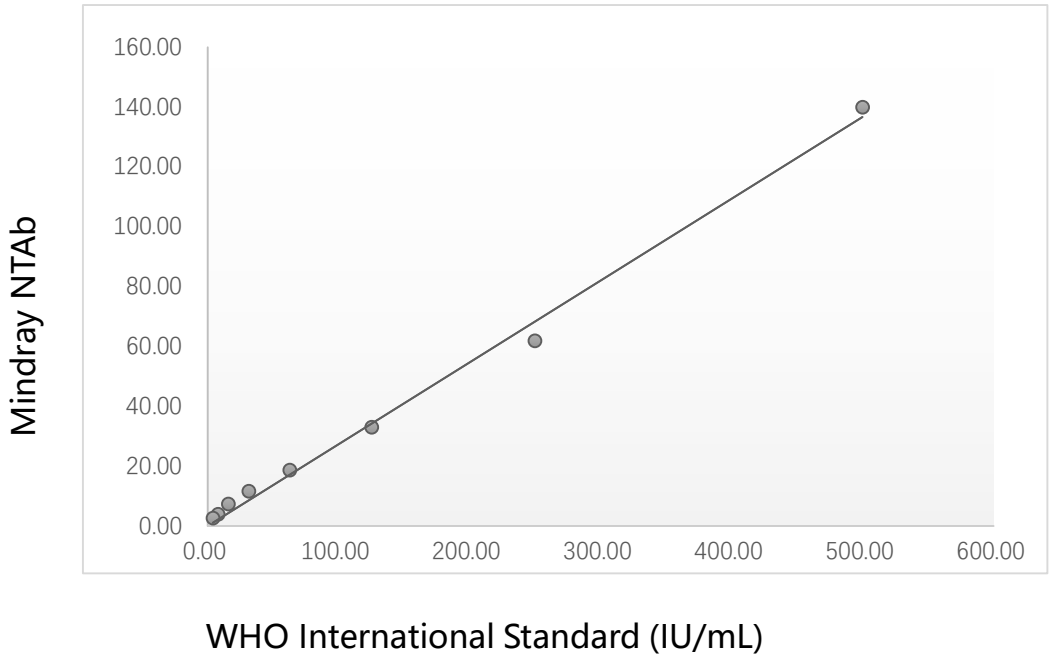
Mindray SARS-CoV-2 S-RBD IgG assay performed good correlation with WHO standard materials.

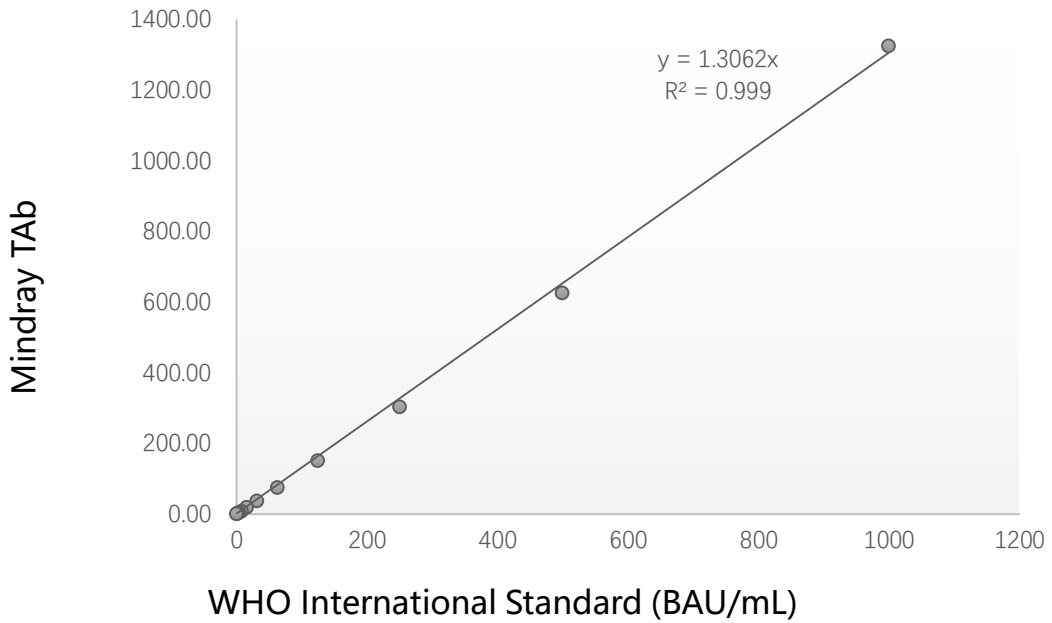
Pearson correlation coefficient： r=0.9978

Conversion： Mindray AU=0.8229×BAU

WHO/BS/2020.2403

WHO Standardization Results of neutralizing antibody and total antibody





NTAb: Pearson correlation coefficient r=0.9975

Conversion : Mindray AU=0.2734×IU

TAb: Pearson correlation coefficient r=0.9995

Conversion : Mindray AU=1.3062×BAU

What can Mindray provide?

- Mindray SARS-CoV-2 **IgM/IgG** assays and
- Mindray SARS-CoV-2 **S RBD IgG** assay

- Intravenous serum or plasma (heparin and citrate), easy to operate;
- Time to first result: **30 minutes**;
- Up to **480 tests/hour** depending on different analyzer models used;
- Fully automatic testing, minimizing infection risk;
- Package: **2 x 50T and 2 x 100T.**

IgM/IgG can support diagnosis of COVID-19.

S RBD IgG can indicate the immune response induced by natural infection and vaccines

CL-900i (180T/h)

CL-1000i /CL-1200i(180T/h)

CL-2000i (240T/h)

CL-6000i (480T/h)

Reference

1. Xia, S. et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. Lancet Infect Dis 21, 39-51, doi:10.1016/S1473-3099(20)30831-8 (2021).
2. Xia, S. et al. Effect of an Inactivated Vaccine Against SARS-CoV-2 on Safety and Immunogenicity Outcomes: Interim Analysis of 2 Randomized Clinical Trials. JAMA 324, 951-960, doi:10.1001/jama.2020.15543 (2020).
3. Zhang, Y. et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 21, 181-192, doi:10.1016/S1473-3099(20)30843-4 (2021).
4. Wu, Z. et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis, doi:10.1016/S1473-3099(20)30987-7 (2021).
5. Jackson, L. A. et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med 383, 1920-1931, doi:10.1056/NEJMoa2022483 (2020).
6. Anderson, E. J. et al. Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. N Engl J Med 383, 2427-2438, doi:10.1056/NEJMoa2028436 (2020).
7. Sahin, U. et al. BNT162b2 induces SARS-CoV-2-neutralising antibodies and T cells in humans. medRxiv, 2020.2012.2009.20245175, doi:10.1101/2020.12.09.20245175 (2020).
8. Walsh, E. E. et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 383, 2439-2450, doi:10.1056/NEJMoa2027906 (2020).
9. Logunov, D. Y. et al. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. Lancet 396, 887-897, doi:10.1016/S0140-6736(20)31866-3 (2020).
10. Folegatti, PM. et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 2020; 396: 467–78, doi:10.1016/S0140-6736(20)31604-4 (2020).
11. Ramasamy, M. N. et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet 396, 1979-1993, doi:10.1016/S0140-6736(20)32466-1 (2021).
12. Sadoff, J. et al. Interim Results of a Phase 1-2a Trial of Ad26.COV2.S Covid-19 Vaccine. N Engl J Med, doi:10.1056/NEJMoa2034201 (2021).
13. Keech, C. et al. Phase 1-2 Trial of a SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine. N Engl J Med 383, 2320-2332, doi:10.1056/NEJMoa2026920 (2020).
14. Yang, S. et al. Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001) against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials. Lancet Infect Dis, doi:10.1016/S1473-3099(21)00127-4 (2021).