TechNotes

HyTest

Clinical and Research Area | Infectious Diseases | Immunology and Serology

Reagents for SARS-CoV-2 Antigen and Antibody Assays

SARS-CoV-2 is a novel coronavirus causing COVID-19. In March 2020 World Health Organization announced the COVID-19 outbreak as a pandemic.

SARS-CoV-2 (see Figure 1) belongs to a large family of single-stranded RNA viruses (+ssRNA). Betacoronaviruses such as SARS-CoVs can cross species barriers and cause in humans illness ranging from a common cold to more severe diseases such as Severe Acute Respiratory Syndorme (SARS, identified in 2003) and Middle East Respiratory Syndrome (MERS, identified in 2012).

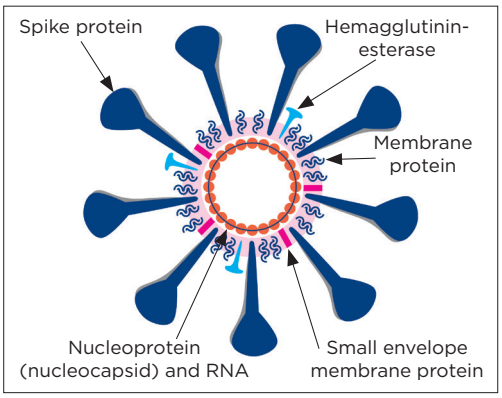

Figure 1. Schematic picture of SARS-CoV-2 virus.

COVID-19 antigen tests

COVID-19 antigen tests are used for detecting the presence of viral antigens in clinical specimens. Several antigen tests specific for SARS-CoV-2 have already been registered to be used for the diagnosis of COVID-19 in the US and Europe, for example.

Whilst tests based on detection of viral RNA are considered as the gold standard in COVID-19 diagnosis, the specificities of the best antigen tests are at par with these RT-PCR assays. Sensitivities are in general somewhat lower, however, since antigen tests are fast and easy-to-use, on several occasions they provide a viable alternative for disease diagnostics and screening purposes.

CLINICAL UTILITY

- ✓ SARS-CoV-2 serology (antibody) tests
- ✓ SARS-CoV-2 antigen tests

COVID-19 serology tests

Serology (antibody) tests are used for monitoring the presence of antibodies specific to SARS-CoV-2 in a clinical sample. During the course of a typical infection, B-cells produce antibodies of different classes. Usually, IgM antibodies can be detected first, whereas IgG class antibodies appear only later (see Figure 2). IgM and IgG antibodies are the most common targets in COVID-19 antibody tests, however, recent studies suggest that measuring the presence of IgA class antibodies could increase the sensitivity of the tests (1,2).

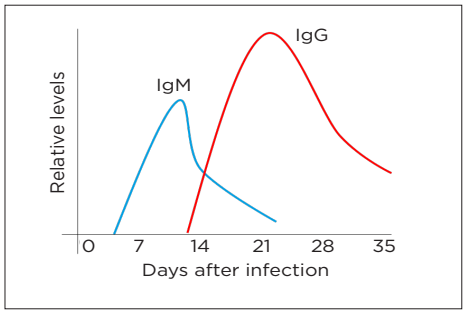

Figure 2. Seroconversion after a typical infection.

References

1. **Yu, H. et al.** Eur Respir J 2001526 (2020) doi:10.1183/13993003.01526-2020.
2. **Tan, C. W. et al.** Nat Biotechnol 38, 1073–1078 (2020).
3. **Ma, H. et al.** http://medrxiv.org/lookup/doi/10.1101/2020.04.17.20064907 (2020) doi:10.1101/2020.04.17.20064907.

Reagents for assay development

We provide several monoclonal antibodies (MAbs) specific to SARS-CoV-2 Nucleoprotein and SARS-CoV-2 Spike. The antibodies are suitable for developing COVID-19 antigen tests. All recommended pairs have been tested with patient samples and/or viral lysates. We recommended to test different antibody combinations as the performance of the assays depends on the platform.

We also offer four recombinant SARS-CoV-2 antigens that can be used in the development of various COVID-19 assays and as positive controls in antigen tests: Spike RBD, two different Nucleoprotein antigens and ACE2-Fc.

In addition, we provide monoclonal antibodies specific to different Ig classes: IgA, IgG and IgM. These can be used as secondary antibodies in serology assays.

Table 1. Cross-reactivity of selected anti-NP antibody pairs to recombinant MERS-CoV Nucleoprotein.

Capture	Detection	MERS-COV NP (His-tag)
C518	C524	-
C518	C706	-
C524	C527	-
C524	C706	-
C527	C715	-
C706	C518	-
C715	C518	-
C715	C706	-

Monoclonal antibodies specific to SARS-CoV-2 Nucleoprotein

We provide several mouse and rabbit derived monoclonal antibodies specific to SARS-CoV-2 nucleoprotein.

Cross-reactivity studies

Several pair combinations have been tested for their cross-reactivity against recombinant MERS nucleoprotein (see Table 1). The same pairs showed no cross-reactivity against several other respiratory disease viruses including seasonal coronaviruses, influenza A and B, human respiratory syncytial virus and adenovirus (see Table 2).

Table 2. Same pairs as in Table 1 were tested for cross-reactivity against several seasonal coronaviruses and other respiratory disease-causing viruses. No cross-reactivities were detected.

Recombinant antigens (from Sino Biological)
Influenza B (B/Florida/4/2006) Nucleoprotein (His Tag) 40438-V08B
Influenza A H1N1 (A/California/07/2009) Nucleoprotein (His Tag) 40205-V08B
Human coronavirus (HCoV-HKU1) Nucleoprotein (His Tag) 40642-V07E
Human coronavirus (HCoV-OC43) Nucleoprotein 40643-V07E
Human coronavirus (HCoV-229E) Nucleoprotein (His Tag) 40640-V07E
Human coronavirus (HCoV-NL63) Nucleoprotein (His Tag) 40641-V07E

Virus lysates
HCoV E229
HCoV OC43
Parainfluenza Type1
Parainfluenza Type2
Parainfluenza Type3
Influenza A (H2N2)
Influenza A (H7N9)
Influenza A (H1N1) pdm09 Guangdong-Maonan
Influenza A (H3N2) HongKong/2671/2019
Influenza A (H5N1)
Influenza B Washington 02/2019
Influenza B Phuket
Human respiratory syncytial virus
Adenovirus

Putative epitope regions

Exact epitope regions have not been determined, however, for the moment we have been able to separate all anti-nucleoprotein antibodies into three epitope groups all within the N-terminal part (see Figure 3). All N-terminal antibodies bind to N47-A173 region of the nucleoprotein. C524, C706, C518, and C715 recognize structural epitopes within the N-terminal part of nucleoprotein. C527 recognizes a linear epitope R89-W108.

Antibodies belonging to different groups are able to form pairs. Further characterisation would be needed to reveal antibodies' true epitope specificities.

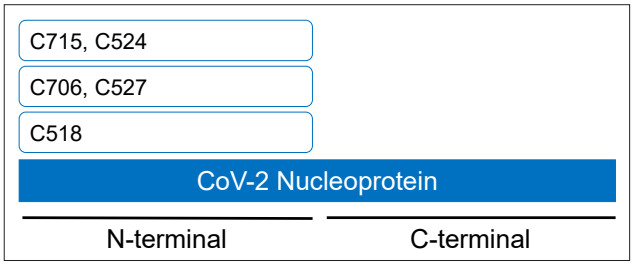

Figure 3. Putative epitope regions of anti-nucleoprotein antibodies. Note that the picture is not a true illustration of the epitopes or their boundaries. It represents our current understanding of how the antibodies form three groups within the N-terminal part.

Pair recommendations

Pair recommendations are listed in Tables 3 and 4. Please note that these are just suggestions based on our internal testing and customer feedback. We continue to collect customer feedback and conduct our own tests and thus, the recommendations are subject to change. It would be important to test several pairs as the performance is dependent on several factors including the platform, buffers, assay conditions etc.

Table 3. Pair recommendations for sandwich immunoassays.

Detection antibody conjugated with HRP:	
Capture	**Detection**
C524	**C706**
C518	**C524**
C524	**C527**
C715	C706
C527	C715

Detection antibody conjugated with biotin:	
Capture	**Detection**
C524	**C706**
C706	**C518**
C715	**C518**
PSN5	**C518**
PSN5	**C524**
C524	C527
C518	C706
C524	C518

Table 4. Pair recommendations for detecting SARS-CoV-2 Nucleoprotein in lateral flow.

1+1		Advanced	
Capture	**Detection**	**Capture**	**Detection**
C715	**C706**	C706	C518+C524
C706	**C524**	C706	C518+C524+C715
C706	**C518**	C706+C518	C524
PSN5	**C518**	C706+C518	C524+C715
PSN5	**C524**	C518+C524	C706
PSN5	**C706**	C518+C524+C715	C706
C518	C706	C524+C715	C706+C518
C524	C706	C518+C524+C715	C706+C527
C706	C715	C706+PSN5	C518+C524

Monoclonal antibodies specific to SARS-CoV2 Spike (RBD)

We provide six MAbs specific to RBD region of Spike 1. Pair recommendations are shown in Tables 5 and 6.

Table 5. Pair recommendations for sandwich immunoassays for detecting SARS-CoV-2 Spike.

Detection antibody conjugated with HRP:	
Capture	**Detection**
RBD5305	**RBD1106**
RBD5308	RBD5305
RBD5324	RBD5308

Detection antibody conjugated with biotin:	
Capture	**Detection**
RBD1106	**RBD5313**
RBD1106	**RBD5305**
RBD5305	**RBD1106**
RBD5308	RBD5313
RBD5308	RBD5305

Table 6. Pair recommendations for detecting SARS-CoV-2 Spike in lateral flow.

Capture	Detection
RBD5308	**RBD5324**
RBD5308	**RBD5313**

Capture	Detection
RBD5324	RBD5308
RBD5313	RBD5308

Clone R107 has neutralising properties

One of the anti-Spike antibodies, clone R107, showed strong neutralising properties in a virus neutralising assay performed according to a test recently described by Tan et al. (3). R107 efficiently inhibited the interaction between recombinant RBD and angiotensin converting enzyme 2 (ACE2) (see Table 7). This interaction has been shown to be critical for coronavirus cell entry.

Note! We do not recommend using R017 in COVID-19 Antigen tests as it does not efficiently detect the South African variant. However, due to its ability to efficiently inhibit ACE2-RBD interaction, it could be used in antibody neutralisation assays as a calibrator.

Table 7. Virus neutralisation experiment with recombinant ACE2 and RBD antigens showed that clone R107 was able to inhibit the interaction between ACE.

Sample	OD	% inhibition
RBD1106	1.4614	22.8
R107	0.0635	96.6
Patient with high titer of neutralising antibodies	0.2	89.4
Negative control	1.9156	-1.2
Negative control	1.8689	1.2
Positive control	0.1465	92.3

Recombinant SARS-CoV-2 antigens

Spike RBD is a fragment Arg319-Phe541 of the spike surface glycoprotein and contains the receptor binding domain of the virus. It has been expressed in mammalian cells and its purity is over 95%. Nucleoprotein is a full-length nucleocapsid expressed in *E. coli*. Purity of the protein is over 95%.

Recombinant human ACE2-Fc

Angiotensin converting enzyme 2 (ACE2) is a cell membrane receptor known to mediate cell entry of SARS-CoV-2 after the virus RBD domain binds to the receptor.

We now have recombinant human ACE2-Fc available (Cat.# 8AE5). The protein consists of the extracellular domain of ACE2 and Fc-fragment of human IgG1 at its C-terminus. It binds to recombinant RBD and could be used e.g. in virus neutralisation assays.

Anti-immunoglobulins for serology assays

We also provide anti-IgM, anti-IgG as well as anti-IgA antibodies suitable for serology tests.

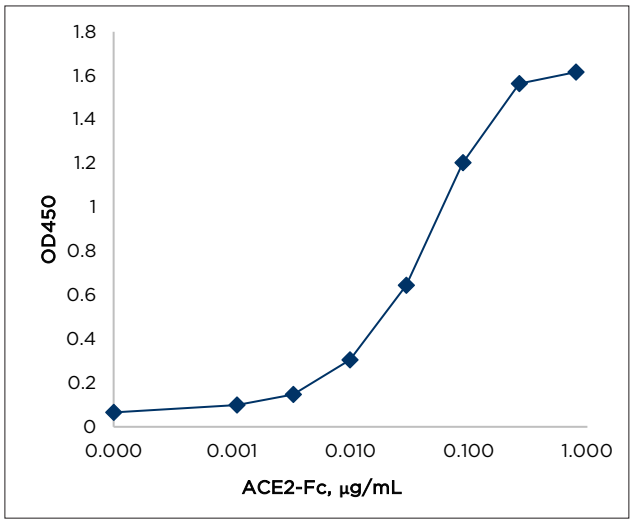

Figure 4. Titration curve of ACE2-Fc shows binding to recombinant RBD coated to microplate wells (1 µg/well).

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Isotype	Remarks
SARS-CoV-2 Spike RBD	3CV2	R107	IgG1	*In vitro,* EIA, ACE2-RBD binding inhibition
		RBD1106	IgG1	EIA
		RBD5305	IgG1	R&D sample, EIA, recombinant chimeric antibody
		RBD5308	IgG1	R&D sample, EIA, recombinant chimeric antibody
		RBD5313	IgG1	R&D sample, EIA, recombinant chimeric antibody
		RBD5324	IgG1	R&D sample, EIA, recombinant chimeric antibody
SARS-CoV-2 Nucleoprotein	3CV4	C706	IgG	EIA, recombinant rabbit antibody
		C715	IgG	EIA, recombinant rabbit antibody
		C518	IgG1	*In vitro,* EIA
		C524	IgG1	*In vitro,* EIA
		C527	IgG1	*In vitro,* EIA
IgA	1A1cc	3B7cc	IgG1	*In vitro,* EIA, PHA, Fc-region
		1H9cc	IgG2b	*In vitro,* EIA, Fc-region
IgG	1G1cc	5A9cc	IgG2a	*In vitro,* WB, ID, Fc-region, Pan γ (Cγ 2 domain), N/cr with IgA, IgM
		3D3cc	IgG2a	*In vitro,* EIA, WB, ID, Fc-region, Pan γ (Cγ 3 domain), N/cr with IgA, IgM
IgM	1M3cc	2B9cc	IgG2b	*In vitro,* WB, EIA, FC, µ-chain, Fc-region

POLYCLONAL ANTIBODY

Product name	Cat. #	Host Animal	Remarks
SARS-CoV-2 Nucleoprotein, polyclonal	PSN5	goat	EIA

ANTIGENS

Product name	Cat. #	Purity	Source
ACE2-Fc, human, recombinant	8AE5	>95%	Recombinant
SARS-CoV-2 Spike RBD, mammalian recombinant	8COV1	>95%	Recombinant
SARS-CoV-2 Nucleoprotein, recombinant	8COV3	>95%	Recombinant
SARS-CoV-2 Nucleoprotein fragment N47-A173, recombinant	8COV5	>95%	Recombinant

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