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).

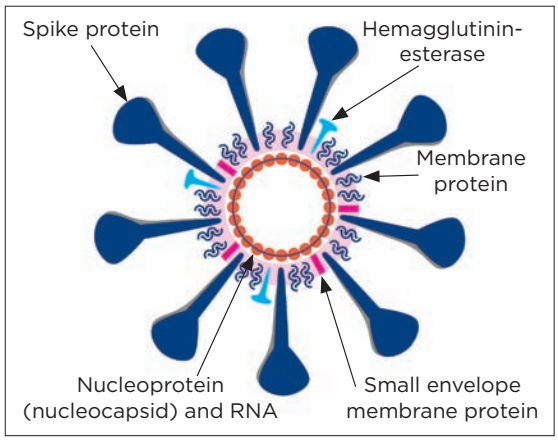

Figure 1. Schematic picture of SARS-CoV-2 virus.

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.

CLINICAL UTILITY

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

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.

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).

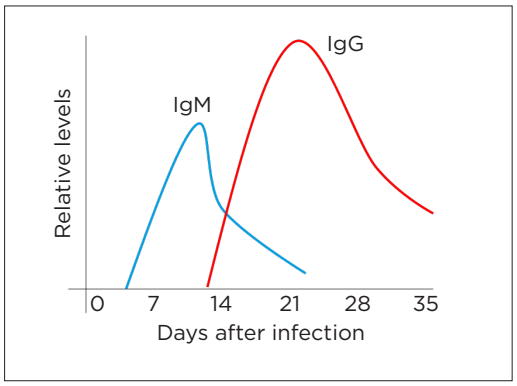

Figure 2. Seroconversion after a typical infection.

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. The final antibody selection has not been decided yet; we continue to characterize different pairs ourselves and we listen to feedback from our customers. We recommended to test different antibody combinations as the performance of the assays depends on the platform. Any clone preferred by our customers will be made available.

We also offer two recombinant SARS-CoV-2 antigens that can be used in the development of COVID-19 antibody tests and as positive controls in antigen tests: Spike RBD and Nucleoprotein.

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)
NP1510	NP1517	-
NP1516	NP1510	-
NP1516	C525	-
NP1517	NP1503	-
C518	C524	-
C518	C706	-
C524	NP1516	-
C524	C527	-
C524	C706	-
NP1526	NP1528	-
C527	C715	-
NP1529	NP1508	-
NP1529	C524	-
NP1529	C525	-
NP1529	C706	-
NP1632	C706	+-
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 five epitope groups, three closer to the N-terminal part and three to the C-terminal part (see Figure 3). Antibodies belonging to different groups are able to form pairs. Further characterisation would be needed to reveal antibodies' true epitope specificities.

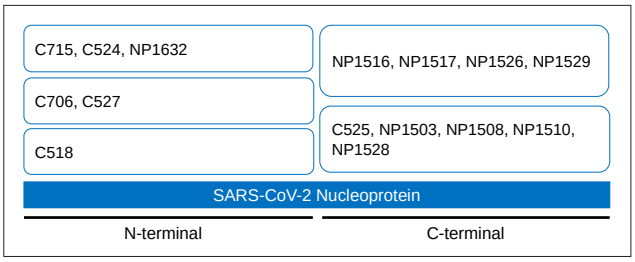

Figure 3. Putative epitope regions of anti-nucleoprotein antibodies. Note that the picture is not a true illustration of the epitopes or their boundaries. It represents our current understanding of how the antibodies form six groups of which three are more N-terminal and three are more C-terminal.

Pair recommendations

Preliminary 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. Preliminary pair recommendations for sandwich immunoassays.

Detection antibody conjugated with HRP:		Detection antibody conjugated with biotin:	
Capture	**Detection**	**Capture**	**Detection**
C524	**C706**	**C524**	**C706**
C518	**C524**	**C706**	**C518**
C524	**C527**	**C715**	**C518**
NP1510	**NP1517**	**NP1510**	**NP1517**
NP1516	**C525**	**NP1517**	**NP1503**
C527	NP1632	**NP1526**	**NP1528**
C715	NP1632	C524	C527
NP1632	C706	C518	C706
C715	C706	C524	C518
C527	C715	NP1529	C524
NP1516	NP1510	C524	NP1516
NP1529	NP1508	NP1529	C706
NP1529	C525		

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

Capture	Detection	Capture	Detection
C715	**C706**	C518	C706
C706	**C524**	C524	C706
C706	**C518**	C706	C715

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. Preliminary pair recommendations for sandwich immunoassays for detecting SASR-CoV-2 Spike.

Detection antibody conjugated with HRP:		Detection antibody conjugated with biotin:	
Capture	**Detection**	**Capture**	**Detection**
RBD5308	RBD5305	RBD5308	RBD5313
RBD5324	RBD5308	RBD5308	RBD5305

Table 5. Preliminary pair recommendations for detecting SASR-CoV-2 Spike in lateral flow.

Capture	Detection
RBD5308	RBD5305
RBD5324	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.

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%.

Anti-immunoglobulins for serology assays

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

References

1. **Yu H, Sun B, Fang Z, Zhao J, Liu X, Li Y, et al.** Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients. Eur Respir J. 2020 May 12;2001526.
2. **Ma H, Zeng W, He H, Zhao D, Yang Y, Jiang D, et al.** COVID-19 diagnosis and study of serum SARS-CoV-2 specific IgA, IgM and IgG by chemiluminescence immunoanalysis [Internet]. Infectious Diseases (except HIV/AIDS); 2020 Apr [cited 2020 May 29]. Available from: http://medrxiv.org/lookup/doi/10.1101/2020.04.17.20064907
3. **Tan CW, Chia WN, Qin X, Liu P, Chen MI-C, Tiu C, et al.** A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nat Biotechnol. 2020 Sep;38(9):1073–8.

Ordering information

ANTIGENS

Product name	Cat. #	Purity	Source
SARS-CoV-2 Spike RBD, mammalian recombinant	8COV1	>95%	Recombinant
SARS-CoV-2 Nucleoprotein, recombinant	8COV3	>95%	Recombinant

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Isotype	Remarks
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
SARS-CoV-2 Spike RBD	3CV2	R107	IgG1	*In vitro,* EIA
		RBD1106	IgG1	EIA
		RBD4319	IgG1	R&D sample, EIA, rat-mouse heterohybridoma antibody
		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
		C525	IgG1	*In vitro,* EIA
		C527	IgG1	*In vitro,* EIA
		NP1503	IgG1	EIA
		NP1510	IgG1	EIA
		NP1516	IgG1	EIA
		NP1517	IgG1	EIA
		NP1526	IgG1	EIA
		NP1528	IgG1	EIA
		NP1529	IgG1	EIA
		NP1508	IgG1	R&D sample, EIA
		NP1632	IgG2a	R&D sample, EIA

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