SARS-CoV-2 Antigens

In December 2019, a new severe respiratory disease emerged in the province of Wuhan, China. Later, a novel coronavirus (CoV) named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2; formerly 2019-nCoV) was identified as the aetiological agent of that disease (COVID-19) (Lake 2020; Gorbalenya *et al.* 2020).

SARS-CoV-2 shares 79% homology with the severe acute respiratory syndrome coronavirus (SARS-CoV) described in 2002/2003 (Lu *et al.* 2020; Zhou *et al.* 2020). Both viruses belong to the family of *Coronaviridae* which are enveloped, positive-sense, single-stranded RNA viruses that can occur in various animal species. Most CoVs affecting humans are causing mild respiratory infections, only the Middle East respiratory syndrome (MERS)-CoV had been reported to be responsible for severe courses of disease besides the SARS viruses.

SARS-CoV and SARS-CoV-2 both use their transmembrane spike (S) glycoprotein to bind the hACE-2 receptor on host cells for cell entry (Tortorici and Veesler, 2019; Zhou *et al.* 2020).

The S glycoprotein is composed of two functional subunits and the S1 subunit mediates binding to the host cell. The S protein is very immunogenic and, as it is mediating binding to the host cell receptor, neutralizing antibodies often target its receptor binding (RBD) domain (Amanat *et al.* 2020). Sequencing revealed that the receptor-binding spike protein encoded by the S gene was highly divergent from other CoVs, with less than 75% nucleotide sequence identity to all previously described SARS-CoVs, except for a 93.1% nucleotide identity to RaTG13, the closest ancestor of the virus occurring in bats (Zhou *et al.* 2020).

The protein sequence of the S gene of SARS-CoV-2 shows three short insertions in the N-terminal domain as well as changes in most of the key residues in the RBD compared with the sequence of SARS-CoV (Zhou *et al.* 2020). This highly diverged motif might be a good target for serological assays that aim to distinguish between the antibody responses against the two SARS virus strains as well as the CoVs causing common cold.

The nucleocapsid (N) protein forms complexes with genomic RNA, interacts with the viral membrane protein and seems to play a critical role in enhancing the efficiency of virus transcription and assembly (McBride *et al.* 2014). Like the S protein, the N protein is highly immunogenic as shown earlier in studies on SARS-CoV (Qiu *et al.* 2005).

In a typical immunological response IgM antibodies are usually the first line of defence. Seroconversion to IgG then occurs later on and these high-affinity IgG antibodies will be responsible long term immunity and immunological memory (Li *et al.* 2020; Racine *et al.* 2009).

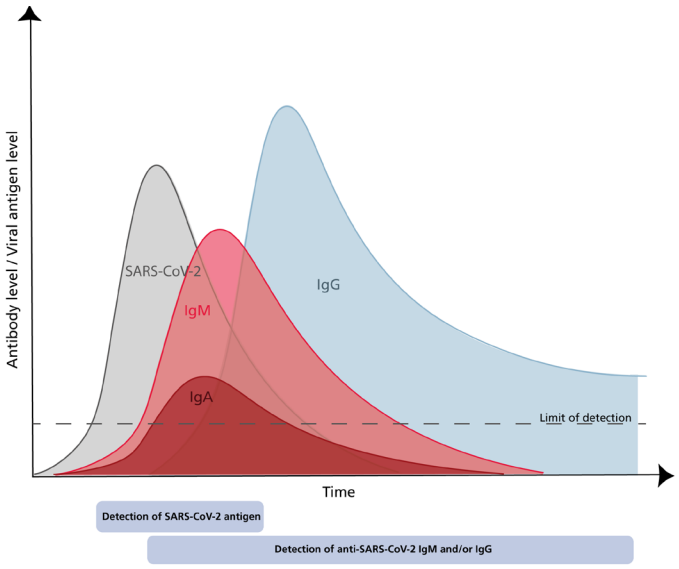
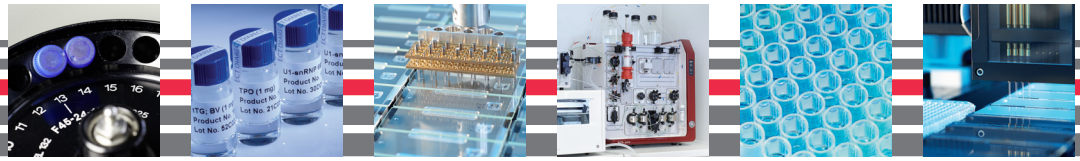


Figure 1: Schematic representation of typical adaptive immune response after viral infection during course of disease and convalescence.

Due to the late onset of antibody development during the course of the disease, molecular methods will be preferred for diagnostics of SARS-CoV-2. However, sensitivity limitations were observed in molecular testing and serology could aid to identify patients appearing false-negative in PCR despite showing symptoms (Farnsworth and Anderson 2020; Xu *et al.* 2020). Further, serology is strongly suggested to play a major role for screening populations to determine exposure as well as potential immunity, to identify convalescent individuals as plasma donors as well as for research on immune response and to help identify neutralizing antibodies (Amanat *et al.* 2020; Farnsworth and Anderson 2020; Okba *et al.* 2020).

A prerequisite of developing highly sensitive serological assays for diagnostic purposes is the use of high quality raw materials that reliably enable distinguishing healthy individuals from those infected. One of the core raw materials of such tests are antigens capable of capturing the patient's antibodies of interest. DIARECT has newly developed SARS-CoV-2 Nucleocapsid (N) Protein and SARS-CoV-2 Spike (S) Glycoprotein outperforming reference antigens (manufactured in different expression systems) in different serological assays. DIARECT's antigens showed a higher reactivity towards patient serum with comparably low background in negative samples at the same time (Figure 2).

Ordering Information		
46000	SARS-CoV-2	0.1 mg
46001	Nucleocapsid (N) Protein	1.0 mg
46100	SARS-CoV-2	0.1 mg
46101	Spike (S) Glycoprotein RBD	1.0 mg



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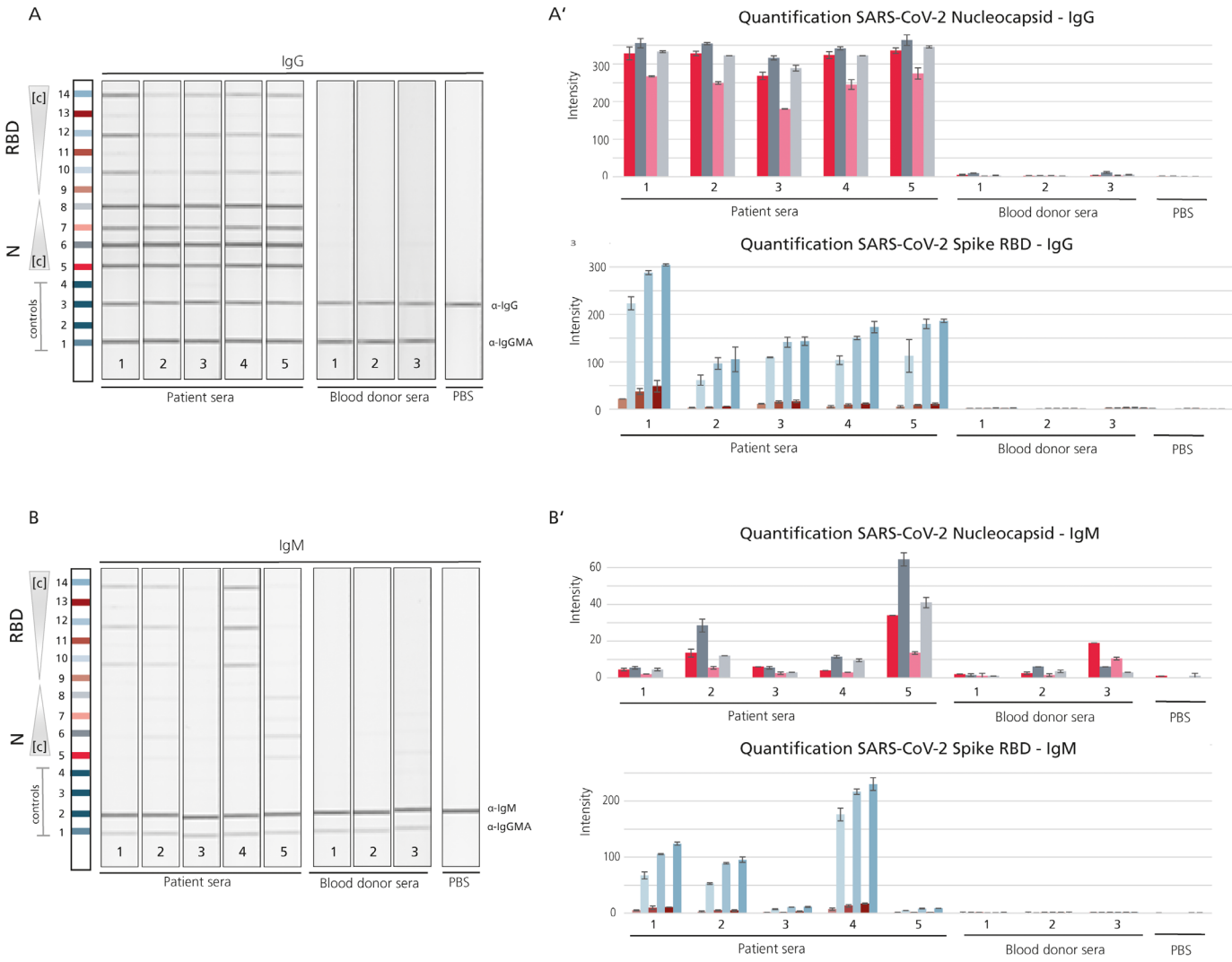

Amount of protein	50 µg/ml	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
	5 Reference N	7 Reference N	9 Reference RBD	11 Reference RBD	13 Reference RBD
	6 DIARECT N	8 DIARECT N	10 DIARECT RBD	12 DIARECT RBD	14 DIARECT RBD

Figure 2: Comparison of DIARECT's Nucleocapsid (N) antigen and Spike (S) RBD antigen to respective reference products.
A; B: Line Assay. The antigens and controls (IgG; IgM; IgGMA) were printed on a nitrocellulose membrane as indicated. Antigens were printed at the following concentrations: Line 5 (Reference N) 50 µg/ml; Line 6 (DIARECT N) 50 µg/ml; Line 7 (Reference N) 25 µg/ml; Line 8 (DIARECT N) 25 µg/ml; Line 9 (Reference RBD) 50 µg/ml; Line 10 (DIARECT RBD) 50 µg/ml; Line 11 (Reference RBD) 75 µg/ml; Line 12 (DIARECT RBD) 75 µg/ml; Line 13 (Reference RBD) 100 µg/ml; Line 14 (DIARECT RBD) 100 µg/ml. Each line assay was incubated with a patient serum (1-5), serum from a healthy blood donor (1-3) or PBS (negative control). Detection was made using anti-human IgG (Figure 2 A) or anti-human IgM (Figure 2 B) secondary antibodies. Each line was prepared in duplicates (only one shown per serum).
A'; B': Graphic evaluation of the line assays. Mean of intensities of line assays (n=2) including standard deviations are displayed.

DIARECT's SARS-CoV-2 antigens are produced in the baculovirus/insect cell expression system.

References:
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