Abstract
AR222, AR249, AS274, AS702 and AS708 antibodies detect by ELISA the spike S protein from SARS-CoV-2.

Introduction
The spike (S) glycoprotein mediates attachment of coronaviruses to the host ACE2 receptor (through the Receptor-Binding Domain [RBD] in the S1 subunit) and fusion with the host cell membrane (through the S2 subunit) (Yan et al., 2020). Here we describe the ability of five recombinant antibodies (AR222, AR249, AS274, AS702 and AS708) to detect by ELISA the soluble ectodomain of the S protein from SARS-CoV-2 (UniProt nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding portion fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker (GGGGS)₃ (see Table 1 for clone names and references) (GeneArt, https://www.invitrogen.com). HEK293 Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 4 days.

Materials & Methods
Antibodies: ABCD_AI334, ABCD_AR222, ABCD_AR249, ABCD_AS273, ABCD_AS274, ABCD_AS702 and ABCD_AS708 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding portion fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker (GGGGS)₃ (see Table 1 for clone names and references). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 4 days.

Results
We tested by ELISA six antibodies recently developed against the SARS-CoV-2 S protein. From these, five (AR222, AR249, AS274, AS702 and AS708) bound in a concentration-dependent manner to the SARS-CoV-2 S protein (Fig. 1). AI334 was used as a positive control (Hammel et al., 2020); AS273 showed no specific binding, most probably due to the fact that this antibody is poorly produced (Table 1, Fig. 1).

Table 1: Clone number, epitope, reference and production yields for the antibodies used in this study.

<table>
<thead>
<tr>
<th>ABCD</th>
<th>Clone</th>
<th>Epitope</th>
<th>Reference</th>
<th>Yield (mg/L)</th>
</tr>
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<tbody>
<tr>
<td>AI334</td>
<td>CR3022</td>
<td>S1</td>
<td>te Meulen et al., 2006</td>
<td>50</td>
</tr>
<tr>
<td>AR222</td>
<td>Sb#14</td>
<td>S1/RBD</td>
<td>Walter et al., 2020</td>
<td>60</td>
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<tr>
<td>AR249</td>
<td>Sb#45</td>
<td>S1/RBD</td>
<td>Walter et al., 2020</td>
<td>100</td>
</tr>
<tr>
<td>AS273</td>
<td>BS6</td>
<td>S1/RBD</td>
<td>Wu et al., 2020</td>
<td>5</td>
</tr>
<tr>
<td>AS274</td>
<td>H4</td>
<td>S1/RBD</td>
<td>Wu et al., 2020</td>
<td>20</td>
</tr>
<tr>
<td>AS702</td>
<td>CV24</td>
<td>S1</td>
<td>Seydoux et al., 2020</td>
<td>20</td>
</tr>
<tr>
<td>AS708</td>
<td>CV30</td>
<td>S1/RBD</td>
<td>Seydoux et al., 2020</td>
<td>20</td>
</tr>
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</table>

Antigen: The prefusion ectodomain (residues 1-1208) of the SARS-CoV-2 S protein, with a KV->PP substitution at residues 986/987, a RRAR->GSAS substitution at residues 682-685, and C-terminal T4 fibrin trimerization motif, protease cleavage site, TwinStrepTag and 8xHisTag (PDB #6VSB; Wrapp et al., 2020), was transiently transfected into 25x10⁸ suspension-adapted ExpiCHO cells (Thermo Fisher) using 1.5 mg plasmid DNA and 7.5 mg of PEI MAX (Polysciences) in 500 mL ProCHO5 medium (Lonza). Incubation with agitation was continued at 31°C and 4.5% CO₂ for 5 days. The clarified supernatant was purified in two steps: via a Strept-Tactin XT column (IBA Lifesciences) followed by Superose 6 10/300 GL column (GE Healthcare) to a final concentration of 180 µg/ml in PBS.

Protocol: S protein (10 µg/ml, 50 µl/well in PBS 0.5% (w/v) BSA, 0.1% (w/v) Tween20) was immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was then rinsed three times with 100 µl of PBS. The reaction was stopped by adding 50 µl per well) for 30 min. After 3 rinses, (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Bio-Rad #170-6516, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H₂SO₄. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Abstract
AR222, AR249, AS274, AS702 and AS708 antibodies recognize the spike S protein from SARS-CoV-2 by ELISA

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References


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Conflict of interest

The authors declare no conflict of interest.

Fig. 1. Specific binding of AI334, AR222, AR249, AS274, AS702 and AS708 antibodies to the SARS-CoV-2 S protein, as detected by ELISA. On the Y axis, ELISA signal (in arbitrary units). On the X axis, the antibody dilution (1:100, 1:1,000 and 1:10,000). ‘S’ refers to the binding to the spike S protein; ‘Ctr’ refers to the binding to biotinylated BSA.