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

Philippe Hammel[1], Anna Marchetti[1], Frederic Zenhausern[2,3,4]

[1] *Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*
[2] *Center for Applied NanoBioscience and Medicine, The University of Arizona, Phoenix, AZ 85004, USA*
[3] *Whitespace Enterprise Corporation, 1305 Auto Drive, Tempe, AZ 85284, USA*
[4] *School of Pharmaceutical Sciences, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*

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 P0DTC2).

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.

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

ABCD	Clone	Epitope	Reference	Yield (mg/L)
AI334	CR3022	S1	ter Meulen *et al.*, 2006	50
AR222	Sb#14	S1/RBD	Walter *et al.*, 2020	60
AR249	Sb#45	S1/RBD	Walter *et al.*, 2020	100
AS273	B38	S1/RBD	Wu *et al.*, 2020	<5
AS274	H4	S1/RBD	Wu *et al.*, 2020	20
AS702	CV24	S1	Seydoux *et al.*, 2020	20
AS708	CV30	S1/RBD	Seydoux *et al.*, 2020	20

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 fibritin trimerization motif, protease cleavage site, TwinStrepTag and 8xHisTag (PDB #6VSB; Wrapp *et al.*, 2020), was transiently transfected into $25x10^8$ 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% CO_2 for 5 days. The clarified supernatant was purified in two steps: via a Strep-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 rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of each antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (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 H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

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).

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Conflict of interest

The authors declare no conflict of interest.

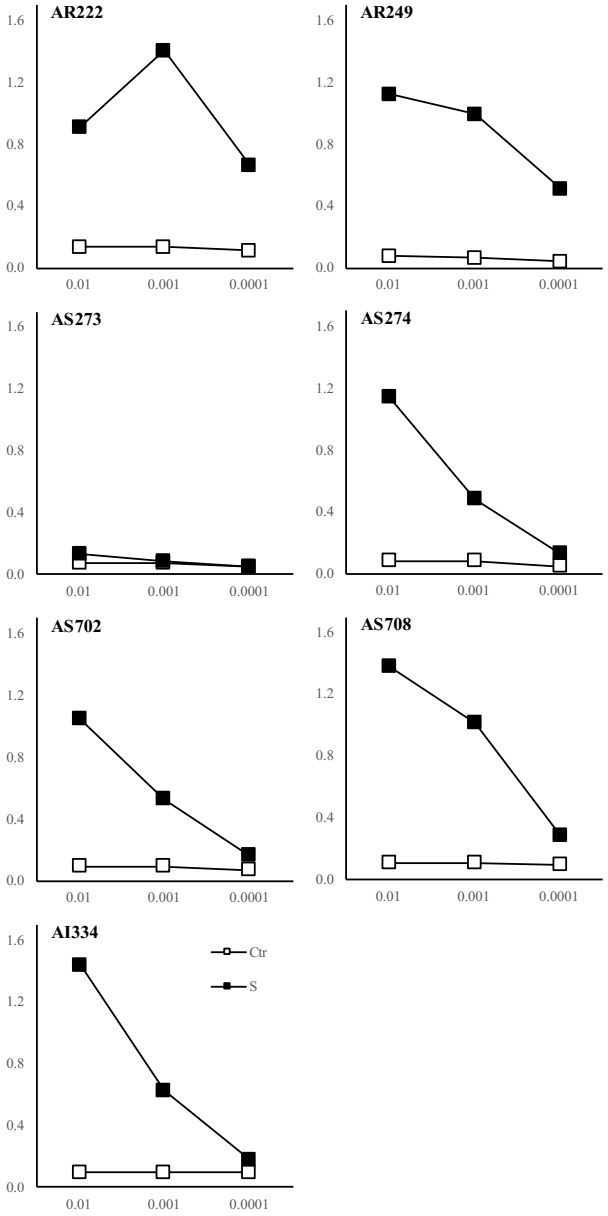

Fig. 1. Specific binding of AI334, AR222, AR249, AS274, AS702 and AS708 antibodies to the SARS-CoV-2 S protein, as detected by ELISA. On the Y axis, ELISA signal (in arbitrary units). On the X axis, the antibody dilution (1:100, 1:1'000 and 1:10'000). 'S refers to the binding to the spike S protein;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