AI334, AQ806, AR222, AR249, AS274, AS708, RB572, RB574 and RB596 antibodies recognize SARS-CoV-2 viral particles by dot blot

Jerome Lacombe[1], Frederic Zenhausern[1,2], Pierre Cosson[3]

[1] *Center for Applied NanoBioscience and Medicine, The University of Arizona, Phoenix, AZ 85004, USA*
[2] *School of Pharmaceutical Sciences, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*
[2] *Cell Physiology and Metabolism Department, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*

Abstract
The recombinant antibodies AI334, AQ806, AR222, AR249, AS274, AS708, RB572, RB574 and RB596 detect inactivated SARS-CoV-2 viral particles by dot blot.

Introduction
SARS-CoV-2, a new member of the Coronaviridae family, emerged in China in December 2019, and has since caused an unprecedented world pandemic (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). Here we describe the ability of nine recombinant antibodies (AI334, AQ806, AR222, AR249, AS274, AS708, RB572, RB574 and RB596) to successfully detect by dot blot inactivated SARS-CoV-2 viral particles.

Materials & Methods
Antibodies: ABCD_AI334, ABCD_AQ806, ABCD_AR222, ABCD_AR249, ABCD_AS274, ABCD_AS708, ABCD_RB572, ABCD_RB574 and ABCD_RB596 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 or VHH 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). HEK 293T suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for the scFv-Fc or VHH-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
AQ806	VHH-72	S1	Wrapp *et al.*, 2020	50
AR222	Sb#14	S1	Walter *et al.*, 2020	60
AR249	Sb#45	S1		100
AS274	H4	S1	Wu *et al.*, 2020	20
AS708	CV30	S1	Seydoux *et al.*, 2020	20
RB572	MRB572	M	Hammel and Zenhausern, 2020	10
RB574	MRB574	M		10
RB596	MRB596	S2	Farrera-Soler *et al.*, 2020	100

Antigen: Inactivated SARS-CoV-2 viral particles were obtained from BEI Resources (NIAID/NIH, NR-52287). They consist of a gamma-irradiated and sonicated cell lysate and supernatant from Vero-E6 cells infected with SARS-CoV-2 (isolate USA-WA1/2020).

Protocol: A droplet of viral particles was deposited (2 µl of undiluted and of 1:5 and 1:25 dilutions) on a 0.45 µm nitrocellulose membrane (Amersham Protran Premium 10600003), and air dried for 5 min. The membrane was blocked for 1 h in PBS containing 0.1% (v/v) Tween20 and 3% (w/v) milk, and washed once for 15 minutes in PBS + 0.1% (v/v) Tween20 (PBS-Tween). The membrane was then incubated overnight (16 h) at RT with the indicated antibodies (final concentration 5 mg/L in PBS-Tween). The membrane was washed three times (15+15+10 min) in PBS-Tween, incubated for 1 h with horseradish peroxidase-coupled goat anti-mouse IgG (Biorad, 170-6516, dilution 1:3000) and washed three times (15 min) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene). All membranes were handled in parallel and exposed together to allow direct comparison of the signal generated by different antibodies.

Results
Antibodies AI334, AQ806, AR222, AR249, AS274, AS708 and, to a lesser extent, RB572, RB574, and RB596 recognize inactivated SARS-CoV-2 viral particles in Vero-E6 cells by dot blot (Fig. 1). An antibody against an irrelevant target (AL626, against the ALFA epitope; Lamrabet, 2020) did not detect any signal (Fig. 1). Two of the antibodies tested here recognize the cytosolic domain of the M protein (RB572 and RB574); the relatively low signal obtained with these antibodies may be due to the fact that the corresponding epitopes are embedded in the virus structure and less accessible to the antibodies.

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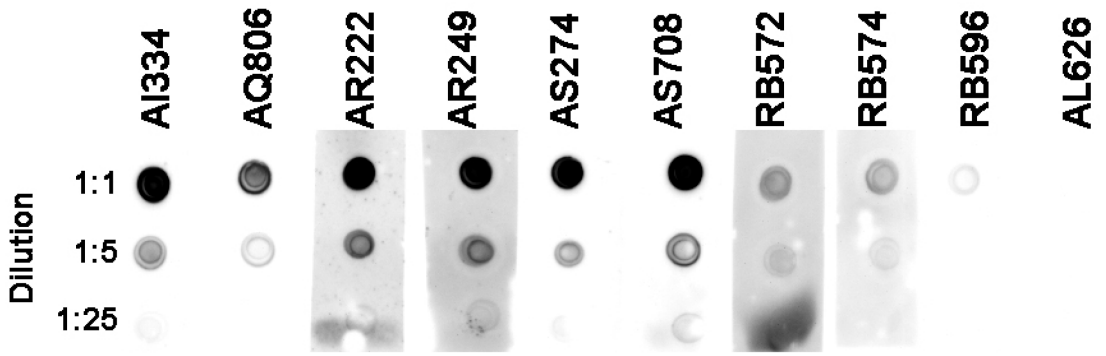

Fig. 1. Antibodies AI334, AQ806, AR222, AR249, AS274, AS708, RB572, RB574 and RB596 recognize inactivated SARS-CoV-2 viral particles in a concentration-dependent manner (concentration of the viral drop deposited: 1:1, 1:5 and 1:25; 2 µl/spot). The negative control antibody AL626 (against an irrelevant target) does not detect any signal.

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Conflict of interest

Pierre Cosson is an editor of the Antibody Reports journal.