The AE889 and AI500 antibodies recognize Klebsiella pneumoniae *surface antigens by flow cytometry*

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Abstract

The recombinant antibodies AE889 and AI500 bind to the surface of the *K. pneumoniae* 52145 strain as detected by flow cytometry; AI144, AI501, AI502, AI505 and AS733 antibodies do not.

Introduction

Klebsiella pneumoniae 52145 strain is a Gram-negative bacterium classified as one of the ESKAPE pathogens, which are drug-resistant and responsible for nosocomial infections (Rice, 2008). Here, we demonstrate the ability of the recombinant antibodies AE889 and AI500 (but not AI144, AI501, AI502, AI505 and AS733) to detect live *K. pneumoniae* 52145 strain by flow cytometry.

Materials & Methods

Antibodies: ABCD_AE889, ABCD_AI144, ABCD_AI500, ABCD_AI501, ABCD_AI502, ABCD_AI505 and ABCD_AS733 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 rabbit IgG2 Fc. The synthesized scFv sequences (Twist Bioscience) 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	Target	References	Yield (mg/L)
AE889	CPS K30 and K33	Goñi *et al.*, 1983	20
AI144	Citrate-sodium symporter	Frey *et al.*, 2008	100
AI500	LPS O1 serotype	Szijárto *et al.*, 2017	90
AI501	K antigen, CPS	Diago-Navarro *et al.*, 2017	30
AI502			20
AI505	LPS O3 serotype	Rollenske *et al.*, 2018	50
AS733	Fimbrial subunit type 3	Wang *et al.*, 2019	70

Antigen: The Kp52145 strain is a clinical *K. pneumoniae* isolate (serotype O1:K2, sequence type 66) (Nassif and Sansonetti, 1986). *K. pneumoniae* 52145 was cultivated overnight at 37 °C in 3 mL of LB medium (Froquet *et al.*, 2009).

Protocol: 1 mL of bacterial culture were centrifuged for 3 min at 4500 rpm. Bacteria were resuspended in 1 mL of SBS buffer (2 mM Na_2HPO_4 $2H_2O$, 14.7 mM KH_2PO_4, 100 mM sorbitol, pH 6.0) and pelleted again. All subsequent steps were performed in SBS buffer. Bacteria were resuspended in 1 mL of buffer and diluted 1/100. 200 µL of diluted bacteria were incubated for 10 min with 2 µg/L of primary antibody at room temperature and under agitation. Bacteria were then centrifuged, washed with 1 mL of buffer, resuspended in 400 µL, then incubated with an Alexa 488-coupled goat anti-rabbit IgG (Life Technologies A-11008, diluted 1/200) for 20 min. Bacteria were washed once with 1 mL of buffer and resuspended in 400 µL before analysis by flow cytometry (BD LSRFortessa Cell Analyzer, 647800E6).

Results

Bacteria incubated with AE889 and AI500 exhibited a clear fluorescent signal compared to negative control, where no primary antibody was used (Fig. 1, No primary antibody). Since Kp52145 bacteria belong to the O1 serotype (Nassif and Sansonetti, 1986), we also used as a negative control AI505, an antibody that recognizes the *K. pneumoniae* LPS O3 serotype (Fig. 1). Antibodies AI144, AI501, AI502 and AS733, as well as the negative control AI505, did not measurably bind live *K. pneumoniae* 52145 (Fig. 1).

The same antibodies were tested against *K. pneumoniae* strains KpGe (Lima *et al.*, 2018) and LM21 (Favre-Bonte *et al.*, 1999), following the same protocol. Antibodies AI144, AI501, AI502, AI505, AS733 and AE889 did not measurably bind KpGe strain. *K. pneumoniae* LM21 incubated with AS733 exhibited a clear fluorescent signal compared to the negative control (no primary antibody), whereas antibodies AI144, AI501, AI502, AI505 and AE889 did not measurably bind the live bacteria (data not shown).

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Conflict of interest

The authors declare no conflict of interest.

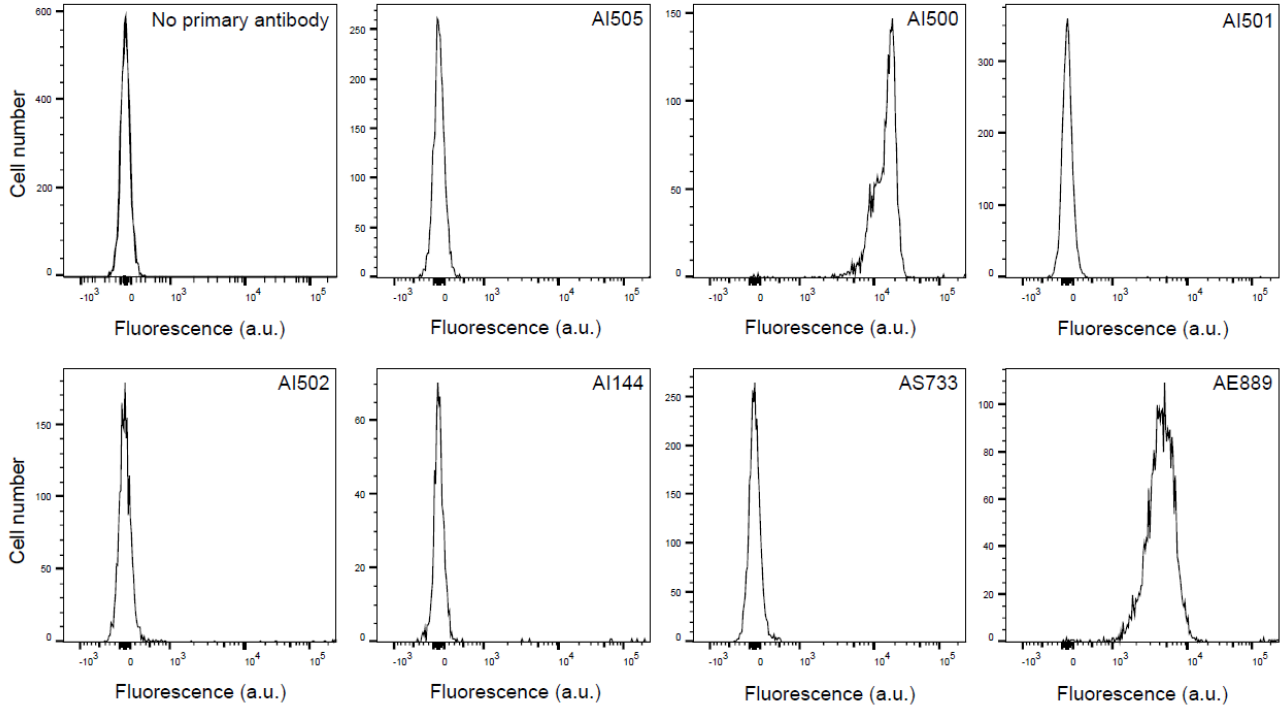

Fig. 1. Live *K. pneumoniae* 52145 are coated with AI500 and AE889, as detected by flow cytometry. Graphs depict the Alexa Fluor 488 signal (Fluorescence axis) vs. the number of events (Cell number axis). AI500 and AE889 bound specifically to Kp52145 bacteria; AI501, AI502, AI144, AS733 and the negative control AI505 did not. No labelling was seen when the primary antibody (No primary antibody) was omitted.