The AE404 antibody recognizes a Pseudomonas aeruginosa *PAO1 surface antigen by flow cytometry*

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Abstract

The recombinant antibody AE404 detects by flow cytometry *P. aeruginosa* PAO1 strain; AE409 and AF389 antibodies do not.

Introduction

Pseudomonas aeruginosa 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 antibody AE404 (but not AE409 and AF389) to bind live *P. aeruginosa* PAO1 strain, as detected by flow cytometry.

Materials & Methods

Antibodies: ABCD_AE404, ABCD_AE405, ABCD_AE406, ABCD_AE407, ABCD_AE409, ABCD_AF389 and ABCD_AQ601 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 (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 production 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 (µg/mL)
AE404	LPS, O5 O-antigen	Emara *et al.*, 1995	40
AE405	LPS, O6 O-antigen		< 5
AE406	LPS, inner core moiety		< 5
AE407	LPS, outer core moiety		< 5
AE409	LPS, O6 O-polysaccharide	Pollack *et al.*, 1995	20
AF389	LPS, O6ad O-polysaccharide	Hemachandra *et al.*, 2001	80
AQ601	SARS-CoV S protein	Coughlin *et al.*, 2007	50

Antigen: The PT5 strain is a wild-type *P. aeruginosa* PAO1 isolate (Köhler *et al.*, 2000). *P. aeruginosa* PT5 was cultivated overnight at 37 °C in 3 mL of SM medium (Froquet *et al.*, 2009).

Protocol: 2 mL of bacterial culture were centrifuged for 3 min at 8000 rpm. Bacteria were resuspended in 2 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 2 mL of buffer and diluted 1/100. A shaken suspension of diluted bacteria (200 µL) was incubated for 10 min with the indicated primary antibody (2 µg/mL) at room temperature. Bacteria were then centrifuged, washed with 1 mL of buffer, resuspended in 400 µL, and incubated with Alexa 488-coupled goat anti-mouse IgG (Life Technologies A11029, 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 coated with AE404 exhibited a clear fluorescent signal compared with negative controls where no primary antibody was used (Fig. 1, No primary antibody) or where both primary and secondary antibodies were omitted (Fig. 1, No antibody).

Antibodies AF389 and AE409, as well as the negative control AQ601 (an antibody that recognizes the SARS-CoV spike protein; Uniprot P0DTC2), did not measurably bind PAO1 bacteria (Fig. 1). AE404 was reported to bind LPS of the O5 group (Emara *et al.*, 1995), while AE409 and AF389 bind LPS of the O6 group (Pollack *et al.*, 1995; Hemachandra *et al.*, 2001). Since PAO1 has been serotyped as belonging to the O5 group (Burrows *et al.*, 1996), it is logical that only AE404 would bind to the bacteria (Table 1).

Antibodies AE405, AE406 and AE407 had low production yields and were not characterized further.

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Conflict of interest

The authors declare no conflict of interest.

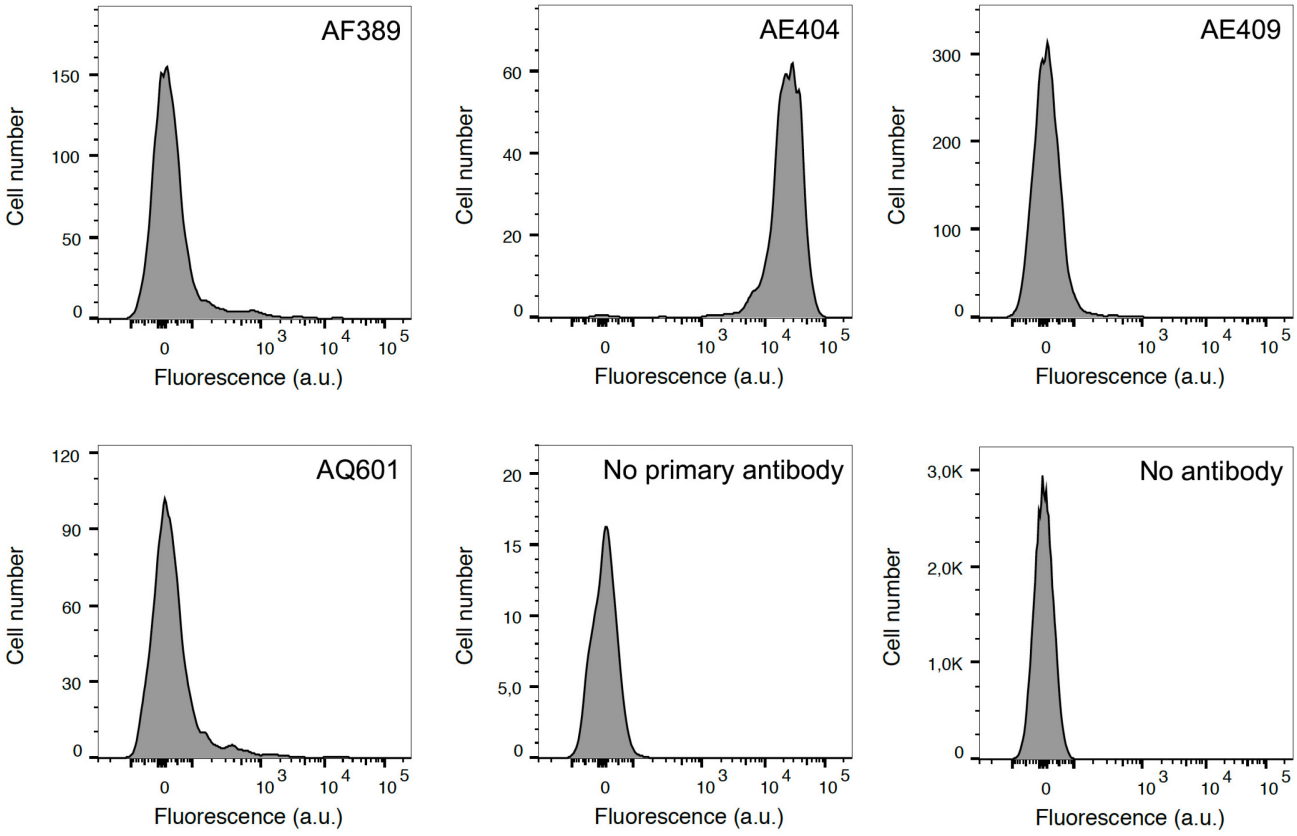

Fig. 1. Live PAO1 *P. aeruginosa* are coated with AE404, as detected by flow cytometry. Graphs depict the Alexa Fluor 488 signal (Fluorescence axis) vs. the number of events (Cell number axis). AE404 bound specifically to PAO1 bacteria; AF389, AE409 and the negative control AQ601 did not. No labelling was seen when the primary antibody (No primary antibody) or the primary and secondary antibodies (No antibody) were omitted.