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Analysis of phage displayed antibody fragment: Pros and cons of phage ELISA and RISE methods

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In-depth characterization of antibodies raised by vaccination is connected with cloning and selecting best binders by hybridoma or display technology. One of gold standards in this approach is antibody phage display technology which can be started from mRNA of immunized animals/people or from hybridoma. Naturally, the abilities of cloned immunoglobulin recombinant fragments (like scFv) to bind antigen of interest, need to be confirmed. Many protocols recommend ELISA with antibody-displaying-phages preparation for such assessment (here reported as standard Phage ELISA), however a quicker solution, named RISE detection, has been also proposed. The cDNA sequence encoding scFv fragment from monoclonal anti-H5 HA antibody (mAb) was cloned into phagemid pSEX81 vector and analysed by Phage ELISA and RISE. In all methods, phages exposing scFv on gpIII capsid protein were incubated with BSA or one of the following H5 HA: Vietnam (A/Vietnam/1203/2004(H5N1)); Qinghai (A/Bar headed goose/Qinghai/12/05 (H5N1)) or self-made (HA₁₇₋₃₄₀) containing H1 region of HA from A/swan/Poland/305-135V08/2006 (H5N1). RISE detection method gave the best results. The mean±SD value of OD₄₅₀ for HA₁₇₋₃₄₀ was 3.34±0.01, for Qinghai it was 4.02±0.03, while the background OD₄₅₀ (for BSA) was 0.31±0.04. Different concentrations of phage preparations and two time periods of incubation of phages with protein (2h and 18 h) were tested, confirming that both, amount of phages exposing scFv and the time of incubation have impact on experiment results.

Biography

Barbara Kalenik has completed her BSc and MSc at University of Warsaw. During studies, she worked in the International Institute of Molecular and Cell Biology, Warsaw, Poland and made 3.5 month Erasmus practice at University of London, School of Pharmacy, London, GB (now University College London). She is a PhD student in IBB PAS group headed by Prof. Agnieszka Sirko. She published one paper in peer-reviewed journal (as Barbara Broniatowska). She was awarded with several scholarships for academic performance by University of Warsaw and Capital City Warsaw and for oral presentation on the 7th Warsaw International Medical Congress.

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