

BIO_19 - Physicochemical characterization of two anti-*Acinetobacter baumannii* monoclonal antibodies

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Introduction: Infections related to health care are considered a world public health problem. The number of registered cases has become a major concern due to the emergence of multiresistant microorganisms and a decrease in antimicrobials development. *A. baumannii* is considered an opportunistic bacterium related to hospital infections that result mostly in pneumonia associated with mechanical ventilation. In recent years, it has been highlighted by the World Health Organization and other regulatory agencies as a pathogen of critical priority for the development of new therapeutic options, due to its broad spectrum of resistance and clinical relevance. In this scenario, non-traditional antibacterials, such as monoclonal antibodies (mAbs), emerge as a highly specific and promising approach.

Objectives: To evaluate physicochemical parameters of two anti-*A. baumannii* monoclonal antibodies such as homogeneity, isoelectric point, secondary structure and thermostability.

Methodology: mAbs that recognize a *A. baumannii* specific surface protein were previously developed by hybridoma technology. In order to evaluate the presence of aggregates and homogeneity of mAbs, Size Exclusion Chromatography (SEC) technique was performed using ÄKTATM Pure System (Cytiva). Evaluation of mAbs isoelectric point (pI) was performed using NuPAGETM Novex isoelectric focusing (IEF) system. Secondary structure profile was evaluated by circular dichroism (CD) in JASCO J-815 spectropolarimeter. Finally, to evaluate the mAbs thermostability, NanoDSF technique was performed using the Prometheus NT.48 equipment.

Results: By SEC analysis both mAbs showed high homogeneity (mAb 1: 99.5%; mAb 2: 98.6%), with only a small fraction of samples with high molecular weight, suggesting the presence of aggregates. Four different protein bands were obtained for mAb 1 with pI distributed between 7.37 and 7.51. For mAb 2 there are also 4 different protein bands, with pI distributed between 7.48 and 7.59. The CD spectra obtained for both mAbs showed a major profile of beta-sheet. In addition, the analysis by NanoDSF suggested mAbs are folded and both lost of secondary structure and aggregation process occurred at temperatures above 50°C.

Conclusion: Considering the results obtained, the two mAbs presented a high homogeneity; they exhibited pI and secondary structure corresponding to what is described in the literature for an IgG isotype antibody; and they are stable at physiological temperatures, serving as a diagnostic or therapeutic tool after humanization.

Keywords: Monoclonal Antibodies; Protein characterization; Antimicrobial resistance