OTR6 - FLUORESCENCE ANALYSIS USED AS QUALITY CONTROL OF MONOCLONAL ANTIBODIES

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Objectives: Proteins often need to be stored for an extended period of time and this fact can lead to loss of their biological activity. Particularly IgG monoclonal antibody shelf life can vary from a few days to more than a year depending of storage conditions. In this context the present work was done aiming to study the stability of an antibody produced against Methicillin resistant *Staphylococcus aureus* (MRSA) using fluorescence analysis.

Methods: The stability of the anti-MRSA was monitored monthly by tryptophan fluorescence emission spectrum. In this sense, the anti-MRSA was incubated at different concentrations (0.2 mg/mL, 0.6 mg/mL and 1.0 mg/mL), temperatures (-70°C, -20°C and 30°C) and pH (6.0, 7.0 and 8.0). The kinetic thermal denaturation of the antibody was also determined in a temperature range interval (25°C-85°C). The excitation wavelength used was 280 nm and the emission wavelength was scanned from 295 nm to 415 nm. Sieving exclusion chromatography was used to evaluate possible aggregation or degradation.

Results: The anti-MRSA samples at pH 6.0 (30°C and -70°C) showed an increase of fluorescence spectra intensity, whereas the samples at pH 8.0 showed a small reduction of fluorescence spectra intensity at 30°C. No differences of fluorescence spectra intensity was observed in the samples at pH 8.0 and -70°C. Fluorescence intensity spectra of samples stored at pH 7.0 and - 20°C did not present alterations. All obtained results did not show shift on maximum emission wavelength or a wide variation in the spectrum area that might indicate a significant conformational change. This observation was confirmed by size exclusion chromatography. The thermal denaturation kinetics of the samples at pH 6.0, 7.0 and 8.0 were seemed. Preliminary studies indicated changes in fluorescence

intensity spectra when the antibody was subjected to extreme pH ($9 \leq pH \leq 4$) and high temperature (≥ 50 °C).

Conclusion: According to spectrum fluorescence analysis of anti-MRSA we concluded that anti-MRSA protein was more stable at pH 7.0 (-20°C).