**ORT_22 - Could antibodies be stable and effective after years of storage?**

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**Introduction:** Monoclonal antibodies (mAbs) are molecules frequently used in diagnosis and therapeutic. The efficacy of mAbs is strongly associated to its stability, which could disturb its potency and safety. Among several factors that could affect conformational integrity and activity of proteins, storage conditions are critical. In this way, evaluating structural and functional stability of mAbs becomes relevant.

**Objective:** To evaluate structural and functional stability of mAbs in different storage conditions.

**Methodology:** Different mAbs ("A", "B", "C" and "D") were produced at Bio-Manguinhos in 2015 and stored at 2-8ºC or -20ºC in phosphate buffered saline. Another batch of mAb "A" was also produced in 2017 and stored at 2-8ºC. Protein concentration was estimated at 280nm and tertiary structure analysis was evaluated by measuring intrinsic tryptophan fluorescence (ITF). Comparative analyses in different storage conditions (2-8ºC and -20ºC) and production time (2015 and 2017) were done. Microscale thermophoresis analysis were performed to verify biomolecular interaction between antigen and mAbs "D" produced in 2015 or 2021 as control.

**Results:** All mAbs analyzed showed similar ITF spectra in both temperature storages. Comparative study of mAbs "A" produced in 2015 and 2017 (stored at 2-8º) showed a shift of >3nm (339-336nm, respectively), indicating changes in conformational structure for the oldest one. Previous results for mAbs "A" produced and analyzed at 2016-2017 showed $\lambda_{max}$ of 332-334nm. When analyzed at 2022 demonstrated $\lambda_{max}$ 336-341nm, suggesting protein denaturation process. mAbs "D" produced in 2015 showed $\lambda_{max}$ of 338-339nm whilst previous data reveled values around 334-336nm, suggesting discrete denaturation over the time. However, termodynamic assay demonstrated similar dissociation constants with antigen compared to 2021 mAbs "D".

**Conclusion:** Results suggested no differences for mAbs stored at 2-8ºC or -20ºC, though changes were observed over time. In despite of discrete changes in conformational structure between mAbs "D" from 2021 and stored one, mAbs are able to interact with their antigen. Complementary experiments have been performed to other mAbs.

**Keywords:** Antibody; Intrinsic tryptophan fluorescence; Microscale thermophoresis