P-048 - STANDARDIZATION OF A HORIZONTAL ELECTROPHORESIS METHOD FOR THE CHARACTERIZATION OF SERUM PROTEINS PRIOR TO DETERMINATION OF GAG’S IN MUCOPOLYSACCHARIDOSIS PATIENTS

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INTRODUCTION: In this work the standardization of the agarose gel electrophoresis (AGE) method was made for the determination of proteins with a “Horizontal System”, between two separation reagents with SDS (sodium dodecyl sulfate) (causes denaturation of proteins) and without SDS; using different reagents such as diluent, buffer charge and developer, allowing to separate the molecules in weight and charge, under conditions of temperature, pH, and voltage. In our university clinical laboratory there are no references for the standardization of electrophoresis

METHODS: With our work it is possible to separate the proteins from the serum before being used for the separation of GAGs and the classification of the different types of Mucopolysaccharidosis. We propose a statistical analysis of the normal distribution between the two reagents.

OBJECTIVES: Standardize the electrophoresis method for the determination of serum proteins, prior to the determination of GAGs for the classification of Mucopolysaccharidosis. Compare two separation reagents with SDS and without SDS.

MATERIALS AND METHODS: Materials; Agarose Gel (2%); (0.5X) pH 8 Buffer (TBE) tris base, boric acid, (0.5M) EDTA; buffer charge, (0.25%) bromophenol blue, (40%) sucrose; (2%) SDS; Samples: blood serum; Method; Horizontal electrophoresis in agarose gel, the equipment was programmed under conditions of 120 V, 1.0 mA, 1h at 20-25oC, according to the User’s Manual. 10μL of samples previously treated with and without (2%) SDS, heated at 95oC for 3 minutes and (40%) sucrose, (0.25%) bromophenol blue, in proportions 1: 1, 2: 1, 1: 2, 1: 3. In the bands: dye solution (0.25%), coomassie blue, (40%) methanol, (10%) acetic acid, was used for 1 hour and was developed with distilled water.

RESULTS: The statistical analysis of normal distribution assumes equal variances with 0.958 (IC ± 1.299) and probability (p > 1.00) using Anderson-Darling, Ryan-Joiner and Kolmogórov-Smirnov, there are no significant differences in the use or not of SDS.

CONCLUSIONS: It is recommended to use for the determination of GAGs and classification of Mucopolysaccharidosis. The statistical analysis creates a support tool that allows determining the optimal conditions of temperature, pH and voltage, the use or not of SDS.