O-018 - DEVELOPMENT OF A HOME-MADE FLUOROMETRIC METHOD FOR THE MEASUREMENT OF BIOTINIDASE ACTIVITY IN DRIED BLOOD SPOTS: PRELIMINARY RESULTS.

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INTRODUCTION: Newborn screening (NBS) for Biotinidase deficiency (BD) is conducted determining the Biotinidase activity in dried blood spots (DBS) using colorimetric assays (quali or quantitative) or fluorometric methods (FM). Colorimetric methods (CM) are the most widespread used. They measure the released p-Amidobenzoic Acid (PABA) from N-Biotinyl-PABA as a purple compound quantifiable at 540nm. FM measures the fluorescence of 6-Aminoquinoline produced by the cleavage of the susbtrate Biotinyl-6-Aminoquinoline, at 355nm/460nm. NBS for BD was implemented in the Fundación Bioquímica Argentina in 1997 using a quantitative colorimetric home-made method (Q-CM), and until Dec/2017, 1,810,521 newborns were screened. ***OBJECTIVE:*** To present the preliminary results of the development of a home-made FM for the measurement of Biotinidase activity in DBS and the comparative results regarding to the in-house Q-CM used in routine. ***MATERIALS AND METHODS:*** Biotinyl-6-Aminoquinoline (Hangzhou Sage-Chemical) 27.0mM was prepared in absolute ethanol. DL-Dithiotreitol (Hangzhou Sage-Chemical) 13.0mM was prepared in potassium phosphate buffer 0.15M, pH=6.5. Biotinidase calibrators in DBS were prepared following an own protocol developed in 1999, and their activities expressed in nmol 6-Aminoquinoline/min.ml-serum, were assigned against a 6-Aminoquinoline (Aldrich) calibration curve. Measurements were made in black 96-microwell plates (Greiner Bio-One) in a Victor2 Fluorometer (PerkinElmer). Control materials were prepared in the laboratory. Newborn samples were obtained from daily routine. Assay protocol: 1/8” discs impregnated with blood were eluted and incubated 4 hrs at 37°C with 50µl of phosphate buffer 0.15M, pH=6.5, containing Biotinyl-6-Aminoquinoline (0.675mM) and Dithiotreitol (1.5mM). The reaction was stopped adding 200µl of absolute ethanol. After 30 minutes, plates were centrifuged and measured at 355nm/460nm. RESULTS AND ***CONCLUSIONS:*** The FM developed resulted analytically and technically reliable and robust. It was linear (r=0.984) in the Biotinidase range evaluated (0.400-5.000) and precise (CV=8.8 y 5.8% at 0.441 y 3.439 nmol/min.ml serum, respectively). It became slightly more expensive than the Q-CM developed in house, however, its technical simplicity (two reagent dispensing steps against five, without needing to transfer supernatants for measurements), and the higher sensitivity of the fluorometric measurements, support its implementation in routine. FM and Q-CM showed an acceptable newborns results correlation (r=0.805), but more data are needed for more significant results.