OPTIMIZATION OF TWO-DIMENSIONAL ELECTROPHORESIS FOR HUMAN CARDIAC TISSUES: VALVE AND MYOCARDIUM.

Francoso, K.S.[1,2]; Teixeira, P.C.[1,2]; Honorato, R.[1] Kalil, J.[1,2]; Guilherme, L[1,2]; Cunha-Neto, E.[1,2]

[1]Laboratório de Imunologia, Instituto do Coração e [2]Disciplina de Alergia e Imunopatologia, Faculdade de Medicina, Universidade de São Paulo, SP, Brasil.

Two-dimensional electrophoresis can separate proteins from complex mixtures and provides information about post-translational modifications. However, to explore all benefits of the technique, a rigorous standardization becomes necessary. The aim of the study was to evaluate several technical conditions on two-dimensional electrophoresis of heart tissues. We analyzed distinct protein loading (cup-loading, paper-bridge and in gel rehydration), and staining (Coomassie blue, deep purple and Cy labeling), using valve or myocardium homogenates. Deep purple staining showed better resolution compared to coomassie staining, since 4x more sensitive for both tissues allowing the visualization of low abundance proteins while Cy labeling was even more sensitive for myocardium since 10x less protein application showed similar number of spots from deep purple staining (1400 and 1624 spots, respectively). The cup loading and paper-bridge applied at the cathode showed improved resolution for myocardium sample (1624 spots), especially of basic proteins. The application of valve sample at the anode revealed 1170 spots, resolved better the high molecular weight proteins and allowed a better isoeletric focusing leading to diminished smears and improved separation of isoforms. The optimal technical conditions for each tissue, valve or myocardium, were distinct due to different characteristics, highlighting the importance of two-dimensional electrophoresis optimization.

Supported by: Fapesp and CNPq.