



EVALUATION OF THE EFFICIENCY OF TWO MEMBRANE METHODS FOR OBTAINING TRANSFER FACTOR FROM HORSE BLOOD

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Introduction. The dialyzable leukocyte extract (DLE) is obtained from lymphoid cell lysate, It includes at least 200 different molecules from 1 to 12 kDa. It contains 8 of the 20 essential amino acids (1). Among these molecules there are a group of peptides with molecular weights (MW) between 3.5 and 5 kDa called Transfer Factor (TF), which has an immunomodulating interspecific activity (2). The TF is indicated in the following diseases: allergies, cancer, or herpes zoster. The TF are polar hydrophilic peptides with acidic parts and two regions: a variable and a constant one. The horse TF has a low probability of transmission of pathogens as a main advantage. It is obtained from white blood leukocyte lysate, The dialysis of TF is performed. Subsequently, the TF peptides are identified. However, there are other useful methods of membrane for purification and concentration of TF.

The aim of this study was to evaluate the efficiency of the method of ultrafiltration and dialysis in isolated and coupled form to obtain a higher concentration of oligopeptides with TF activity.

Methods. The horse blood samples were obtained by aortic puncture. Subsequently, each sample was tested the detection of antibodies against *Brucella* by the Bengal Rose method. The cell pellet was obtained by sedimentation of *Brucella* negative samples. Then, leukocyte fraction was obtained by aspiration and concentrated by centrifugation. The leukocyte extract was obtained by thermal shock. Dialysis, Ultrafiltration, Dialysis-Ultrafiltration and Ultrafiltration-Dialysis were evaluated as TF concentration methods. The criteria efficiency of each methods were evaluated by protein concentration by the Lowry method and the electrophoretic patterns by SDS-PAGE technique.

Results. From twelve horse blood samples which were evaluated by antibodies against *Brucella* detection, ten were negative. The

ultrafiltration-dialysis obtained sample was the higher in protein concentration, followed by the one which was obtained by dialysis (see Table 1).

Table 1. Protein content of samples obtained by four concentration methods by TF

Method	Protein concentration (µg/mL)
A. Human TF (pattern)	160.84
B. Diálisis	220.00
C. Ultrafiltración	115.80
D. Diálisis-Ultrafiltración	78.20
E. Ultrafiltración-Diálisis	376.84

However, the sample processed by the dialysis method preserved the protein fractions compared to the human TF (pattern). And the samples processed by ultrafiltration preserved only the lowest MW peptides (see Figure 1)

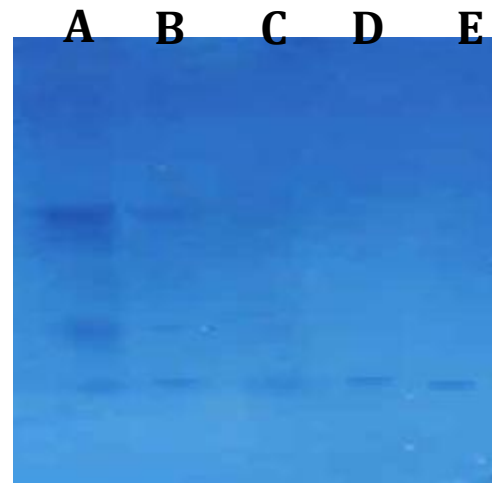


Figure 1. Comparison of four concentration methods for TF by SDS-PAGE.

Conclusion. The ultrafiltration-dialysis method was a higher concentration of protein. However, the dialysis method had an electrophoretic pattern more similar compared to the human TF pattern.

References.

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