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COMBINATION OF T-GEL CHROMATOGRAPHY AND IMMUNOAFFINITY AS A NEW PROTOCOL TO REMOVE IMMUNOGLOBULINS AND ALBUMIN FROM SERUM SAMPLES: THE CASE OF RHEUMATOID ARTHRITIS

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Quantitative analysis of proteins in serum is essential to find disease biomarkers, and 2-DE is still a unrivalled tool to measure quantitative differences. However, the high dynamic range of proteins in serum (up to 12 orders, which exceeds the dynamic range of 2-DE), the fact that only 1% of total serum protein may contain potential biomarkers, and the reproducibility issue, are all still important aspects to solve. Removal of high-abundance proteins is a common practice, but also one of the biggest challenges. Most of the depletion methods used have a low loading capacity, are costly or need equipment not always available at a lab. Thereby, the present work describes a new approach, where depletion of most of immunoglobulin (Ig) isotypes as well as albumin (HSA) from 1 ml serum samples is achieved by using the combination of two methodologies: a thiophilic chromatography, not previously used in 2-DE, and a HSA-specific immunoaffinity resin, respectively. Samples were analysed by 2-DE to evaluate the effectiveness of the depletion method and a pattern of serum proteins, most of them identified by MS, detected. Reproducibility was also assayed, and a preliminary study with rheumatoid arthritis (RA) patients carried out to test the performance of the new protocol.

RA is a chronic autoimmune disease characterized by inflammation at the synovial joint and infiltration of leukocytes. RA treatments are nowadays more effective, but should be started as early as possible; i.e., new RA biomarkers are necessary. We have analyzed Igs/HSA-depleted sera obtained from 3 healthy individuals and 3 recently diagnosed and untreated RA patients (18 gels), finding significative changes in many negative or positive acute-phase proteins previously described as altered in RA (e.g., transferrin or ApoA1). However, SAP, α1-microglobulin or VSP28 have also come up during this preliminary study as proteins upregulated during RA.