Chapter 1 : Identification of Human Plasma Proteins as Major Clients for the Extracellular Chaperone Clust

The depletion of the immunoglobulin bands is more difficult to discern than the depletion of the very abundant albumin band shown in figure 1, but the eluted protein should be clearly seen as two bands representing the heavy and light chains of the immunoglobulin (Ig H and Ig L).

Mean depletion efficiency SD across 4 columns for 21 cycles of albumin depletion. This analysis allowed for a comparison of the downstream effect of ineffective vs effective albumin depletion on peak data. This result is consistent with the poor efficiency of albumin depletion with column 4 throughout the experiment and with the other 3 columns beyond the cutoff point of 17 runs. Open in new tab Figure 2. The mass spectra 48 to 85 kDa for highâ€”laser intensity readings is shown for albumin-depleted serum from column 1 and column 4 for runs 1, 8, 17, and Column 1 was no longer effectively depleting albumin boxed peak by run 20, and column 4 was ineffectively depleting albumin throughout the experiment. For serum depleted by column 1, the mass spectra are reproducible from run to run until run The larger albumin peak seen with column 1, run 21, and with multiple runs from column 4 corresponds to decreased albumin depletion efficiency. The absolute number of peaks described above is relatively low owing to the stringency of the criteria we used to pick peaks see Materials and Methods. We used these criteria to evaluate unambiguous peaks that should be readily reproducible. View popup Table 1. For bioprocessor 1, the mean CV was For bioprocessor 2, the mean CV was The mean CV for specimens from all 3 columns and runs 1, 8, 17, and 21 was These data suggest that neither chip-to-chip nor bioprocessor-to-bioprocessor variability contributed significantly to preanalytical biases, and most likely, inefficient depletion was the source of the lack of reproducibility of SELDI-TOF MS data from run 21 compared with the other 3 runs. Discussion In searching for low-abundance proteins that may be biomarkers of disease, prefractionation of biologic samples by methods such as albumin depletion has become a necessary step for a variety of proteomic methods. Because proteomic profiling involves processes in which the components being measured and their respective quantitative relationships are not known, rigorous quality assurance is necessary to ensure reliability of data Any prefractionation process involves an additional step that could potentially affect the reliability of the data obtained via proteomic techniques, and the potential preanalytical bias caused by this process must be critically evaluated before a technique is accepted as standard practice. Other studies have addressed aspects of QC in the depletion of highly abundant proteins. They found this method to be specific and reproducible based on analysis with protein assays, 2-dimensional gel electrophoresis, and liquid chromatography-tandem MS of proteins excised from gels. They also found that 1 spin column was recyclable for up to 20 different column runs. These investigators, however, assessed the repeatability of SELDI-TOF spectra by analysis of the same serum sample 8 times in 1 day, and reproducibility by analysis of the same sera on 4 consecutive days. Thus, their findings may not mirror real-world proteomic laboratory conditions, in which albumin depletion and proteomic profiling methods are often performed over a series of experiments spanning several weeks or months. Although automated HPLC-based immunodepletion is likely more reproducible, we used spin columns, which are widely used for immunodepletion because of their ease of use and setup. Potential problems that could contribute to variable and decreased spin column lifespan include variations in sample and bead mixing or sample pipetting, or variations in the beads themselves. Visible loss of microbeads into the eluate occurred near the point at which 3 of the 4 columns stopped efficiently depleting albumin. This result suggests that when visible column breakdown occurs during manual depletion of albumin through a spin column, these columns should be discarded and new columns used. We have shown that inefficient depletion leads to mass spectra that differ from those obtained with efficient depletion and that failure of effective albumin depletion cannot always be predicted without calculation of efficiency of the depleted protein. We used the relative intensity of the top 10 peaks that were picked by stringent criteria to examine reproducibility, because these peaks are most likely to be reproducible. Lack of reproducibility of these peaks suggests that low-abundance biomarkers, which would presumably be more susceptible to small changes in sample preparation, are more affected by variability in albumin depletion. Thus, QC processes are necessary for manual immunodepletion before proteomic methods because the manual spin-column lifespan is variable and downstream data are less reproducible after inefficient depletion. We describe quantitative albumin concentration calculation as a reasonable method to evaluate column breakdown. Such a quality assurance measure is not time-consuming or cost prohibitive when 1 highly abundant protein is depleted. The use of IgY columns to deplete multiple highly abundant proteins is widespread, however, and although reproducibility of depletion should be verified in experiments using such columns, QC is much more difficult. In such cases, quantitative albumin concentration may still be a useful QC measure, because changes in albumin concentration would suggest lack of reproducibility in the depletion of the other highly abundant proteins. Therefore, variation in depletion efficiency has a substantial impact on the protein composition of immunodepleted serum. Our finding of peaks unique to the albumin-bound fraction suggests that depletion of albumin changes the serum proteome such that the albumin-bound fraction should not be discarded, but rather further evaluated for unique peaks. Although albumin binds peptides such as insulin and bradykinin 31 , the extent of this peptide binding is unknown Exploration of the albuminome is an evolving area of research interest Ashaunta Tumblin for help with the experiments and Debra Reda for technical support in figure production.

Chapter 2 : - NLM Catalog Result

We report the specific removal of 98% of albumin and 80% of immunoglobulin heavy chain from human plasma by affinity chromatography, and the subsequent improvement in the number of spots detected and their resolution following two-dimensional gel electrophoresis.

Polyclonal antibodies to a variety of plasma proteins were coupled to a matrix by binding to immobilized protein G for goat antibodies or protein A for rabbit antibodies and the complexes stabilized by covalent crosslinking with dimethysuberimidiate. This approach is more efficient that the use of a multiple immunoaffinity columns and does yield a cleaner sample for two-dimensional gel electrophoresis. These investigators found that ultrafiltration, precipitation with trichloroacetic acid or acetone was useful for the preparation of samples. They also observed that ammonium sulfate precipitation could be useful as it could separate albumin; however, other proteins are also likely removed. A proactive protocol for sample processing should be developed and used in the study. Samples should be obtained at the same time to avoid possible complications from diurnal or circadian variation. A stability study is recommended after initial studies. If plasma is used, EDTA is the recommended as the anticoagulant and an absolutely uniform process established for obtaining plasma. It is possible that a sample could be stored as a reference sample but not as a primary sample. Normal values for potential biomarkers must be established prior to the experimental series; it is recommended that 30 normal samples be used. The individual investigator will need to establish whether normal samples need to be age-matched, gender-matched, etc. Correspondence to Roger L. What to do with "one-hit wonders"? Electrophoresis 25, , Analysis by multidimensional separation coupled with mass spectrometry, Mol. Proteomics 1, , Koepke, Churchill Livingstone, Vol. Keopke, Churchhill Livingstone, Vol 2. Breast Cancer 2, , Markers 17, , Effects of time and temperature on 22 serum analytes, Eur. Acta , , Effects on analytical performance of selected serum and plasma hormone assays, Clin. A nonredundant list developed by combination of four separate sources, Mol. Proteomics 3, , College Cardiology 44, , Sample prefractionation via multicompartment electrolyzers with isoelectric membranes, Electrophoresis 21,, Experimental evaluation in a multicompartment electrolyzer, Proteomics 4, , Acta 49, , Ahmed, N, Barker, G. USA 72, , A tool for general affinity chromatography, in Theory and Practice in Affinity Techniques, ed. Eckstein, Academic Press, London, pps. Comparison of different approaches for albumin and gamma-globulin depletion, J. A , , An innovative step towards a comprehensive survey of the human plasma proteome, Proteomics 3, , Analysis of protein expression in toxic oil syndrome studies, Proteomics 4, , Methods , ,

Chapter 3 : Albumin and immunoglobulin depletion of human plasma.

Albumin and Immunoglobulin Depletion of Human Plasma Jenkins, Rosalind E.; Kitteringham, Neil R.; Greenough, Carrie; Park, B. Kevin Summary Plasma and serum have been the focus of intense study in recent years in the expectation that they will provide important biomarkers of health and disease, without the need for invasive.

Human plasma and serum represents an important biological m The global composition of proteins in the blood plasma represents the However, the wide dynamic range in protein concentration remains a major challenge in the development of diagnostic assays for the very low concentration of biomarker proteins in the presence of high abundance proteins. Among a number of plasma protein depletion techniques, the ProteoPrep 20 represents the most powerful enabling technology currently available. Blood plasma is not only the most studied among biological fluids, but also the primary material for disease diagnosis. Blood plasma contains a very high concentration of proteins, typically in the range of mg of protein per ml. Estimates of the number of proteins in blood plasma start from 10,, but the actual number of distinct proteins may be several orders of magnitude higher [1,2]. This is because each protein has a potential for a variety of post-translational and metabolic modifications [], both in normal and diseased cells. The global composition of proteins in the blood plasma represents the plasma proteome. Perfusion of blood through the different organs and tissues can result in the addition of new proteins, removal of some proteins, or modification of existing proteins, which may vary according to specific physiological or pathological conditions []. It is logical to expect correlation between the proteomic profiles of ed the number of proteins analyzed and identified, some of the proteins found in the non-depleted serum were not found in the depleted serum [70,,]. This is mostly attributed to the so-called sponge effect, where small proteins and peptides may bind to large proteins that normally serve as their carriers [,]. In reality there is no quantitative data to show how much of the non-targeted proteins are non-specifically bound to the specifically depleted proteins, and how much are bound to the depletion matrices. Nevertheless, these observations raise concerns about the validity of the quantitative representation of the whole proteome when only the protein-depleted sample is analyzed. Therefore, for particular applications the specifically depleted bound fraction may also be analyzed to ensure that no important proteins are inadvertently omitted. The classical depletion strategy for albumin involves the use of the hydrophobic dye Cibacron blue, a chlorotriazine dye which has high affinity for albumin [,,]. This strategy of removing albumin is still sometimes used in proteomic analyses because of its relatively low cost [52,]. Other small molecules have been designed e. As a group, the immunoglobulins represent the second most abundant proteins in the plasma or serum. A low cost depletion kit for simultaneous depletion of albumin and immunoglobulins Cat. Because of this demonstrated specificity, the trend is now towards the use of immunoaffinity media for most proteomic analyses. Affinity media are made up of matrices with covalently attached antibodies to the specific abundant proteins [15,]. For example, it was estimated that even if In addition, there are still many other highly abundant proteins that can potentially mask the analysis of the low abundance proteins and should, therefore, be removed. For example, columns containing affinity ligands for the top six abundant proteins have been shown to improve the visualization, detection and identification of more low abundance proteins [38,70,73,74, ,,,,], when compared to depletion of only HSA and IgGs. In addition, data from the HUPO Plasma Proteome Project clearly showed that depletion of the most abundant proteins in serum, whether only albumin, albumin and IgGs, or the six most abundant proteins, improved detection of some of the low abundance proteins [80]. However, the same report also indicated incomplete sampling of proteins is a dominant feature. Part of the reason is likely the limitation in the amount of sample that can be loaded for analysis, before the remaining high abundance proteins interfere with the analysis. An affinity column designed to remove the 12 most abundant proteins is also available, but experimental data on this product is yet to emerge. A new affinity column with high binding capacity has been developed. The ProteoPrep 20 Plasma Immunodepletion Kit PROT20 is the only commercially available product that contains immunoaffinity ligands designed to remove 20 of the abundant proteins Table 1 in human plasma or serum []. This novel technology is the most power ful tool currently available, and has demonstrated the ability to deplete more proteins to visualize low copy number proteins in plasma samples and subsequently identify them by mass spectrometry []. For convenience, the ProteoPrep 20 Plasma Immunodepletion Kit PROT20 is supplied as a complete kit containing 3 spin columns and the necessary reagents and consumable supplies. The kit also includes protocols that have been optimized for specific applications. Carefully controlled tests [] indicated that each spin column removed the 20 high abundance proteins with an average depletion of This depletion enabled a fold and a 3-fold increase, respectively, in the load of low abundance proteins compared to the sample without depletion and depletion of just 6 proteins. This enrichment consequently enabled the identification of several low abundance proteins that could not be detected either in the non-depleted serum nor the 6-protein depleted serum. Finally, the spin columns have high economic value because they are re-usable for at least times. As indicated previously, protein depletion can be considered an initial dimension in orthogonal protein separation, the purpose of which is to separate the highly abundant proteins from the low abundance proteins. Since the flow through from ProteoPrep 20 spin column low abundance proteins and the fraction derived from the proteins bound to the affinity media high abundance proteins are both in solution phase, they are amenable to subsequent protein separation steps. A variety of possible combinations of orthogonal protein separation techniques are shown in the workflow Figure 1 , depending on the application and instrumentation available to the researcher. Finally, the different fractions from the different multi-dimensional separation techniques are subjected to trypsin digestion and analyzed by LC-mass spectrometry. Multi-dimensional analysis and mass spectrometry will be discussed separately elsewhere. Typical workflow for protein depletion using ProteoPrep 20 Plasma Immunodepletion Kit PROT20 , leading to multidimensional separation, mass spectrometry, and protein identification. The different separation techniques are enclosed in a dotted box to indicate that any combination of these techniques can be used in an orthogonal manner. Is proteomics heading in the wrong direction? Han KK, Martinage A: Possible relationship between coding recognition amino acid sequence motif or residue s and posttranslational chemical modification of proteins. Post-translational chemical modification s of proteins. Post-translational chemical modifications of proteins--III. Current developments in analytical procedures of identification and quantitation of post-translational chemically modified amino acid s and its derivatives. Int J Biochem , Mann M, Jensen ON: Proteomic analysis of post-translational modifications. Nature Biotechnology , Petricoin E, Liotta L: The vision for a new diagnostic paradigm. Clinical applications of proteomics. Therapeutic potential of the plasma proteome. Rarity gives a charm: Proteomics techniques and their application to hematolo gy. Analysis of the Human Serum Proteome Clin. The human plasma proteome: A non-redundant list developed by combination of four separate sources. Proteomic-based detection of urine proteins associated with acute renal allograft rejection. Development of a novel proteomic approach for the detection of transitional cell carcinoma of the bladder in urine. A catalogue of human saliva proteins identified by free flow electrophoresis-based peptide separation and tandem mass spectrometry. MS Characterization of multiple forms of alpha-amylase in human saliva. Proteomic characterization of the interstitial fluid perfusing the breast tumor microenvironment. Explorative study of the protein composition of amniotic fluid by liquid chromatography Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Diagnosis of intra-amniotic infection by proteomic profiling and identification of novel biomarkers. Rapid detection of fetal aneuploidy using proteomics approaches on amniotic fluid supernatant. Prenatal Diagnosis , The amniotic fluid cell proteome. Identification of new proteins in follicular fluid of mature human follicles. The platelet microparticle proteome. Human tissue profiling with multidimensional protein identification technology. Initial sequencing and analysis of the human genome. The sequence of the human genome. International Human Genome Sequencing Consortium: Finishing the euchromatic sequence of the human genome. Anderson L, Seilhamer J: A comparison of selected mRNA and protein abundances in human liver. A sampling of the yeast proteome. Correlation between protein and mRNA abundance in yeast. The missing beat in biotechnology? A wide range of protein isoforms in serum and plasma uncovered by a quantitative intact protein analysis system. Two dimensional electrophoresis resources available from ExPASy. Clinical applications of proteomics: Use of proteomic patterns in serum to identify ovarian cancer. Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. A recent extensive compilation of human plasma proteins indicated that most of the major categories of proteins in the human body were represented in the blood plasma [15]. Thus, the plasma proteome is an ideal source of diagnostic markers and therapeutic targets for many human diseases [10,11,15]. A protein, or most likely a set of proteins, that undergo changes in concentration or structural composition e. PTM as a result of disease or physiological state can potentially be used as diagnostic biomarkers. A biomarker is an identified protein or group of proteins, which change in concentration or structural composition due to a particular disease state. When blood is coagulated and centrifuged, a translucent liquid called serum separates as a top layer. The coagulated portion is presumed to be mostly fibrin and other proteins involved in the coagulation process. The serum still contains a very high concentration of proteins.

Chapter 4 : Publications Authored by Rosalind E Jenkins | PubFacts

Plasma and serum have been the focus of intense study in recent years in the expectation that they will provide important biomarkers of health and disease, without the need for invasive procedures.

The results shown are representative of three independent experiments. One potential impact of these stresses is damage to proteins, inducing misfolding, loss of function, and aggregation. Although much is known about intracellular mechanisms that act to repair or dispose of stress-damaged proteins, little is known about corresponding extracellular mechanisms. When comparing the intracellular and extracellular environments, some stresses that can contribute to protein unfolding are greater in the latter. The extracellular environment is also more oxidizing than the cytosol Relative to plasma left stationary at room temperature, plasma stressed in this way for 10 days showed increased turbidity and protein precipitation Fig. This result indicates that plasma proteins are prone to unfolding and aggregation under conditions of temperature and shear stress that are likely to occur in vivo. If this is the case, then intuitively, the human body must have systems in place to control this problem. SEC indicated that the level of soluble protein remaining in stressed plasma was less than in batch-matched control plasma and that proportionately more proteins were present as HMW species in stressed plasma Fig. These findings may be explained by the stress-induced partial unfolding of plasma proteins, which subsequently aggregate to form increasingly large aggregates, some of which eventually become too large to stay in solution and form insoluble precipitate. Superimposed on this process, we suggest that under these conditions CLU forms large soluble complexes with probably many different misfolded plasma client proteins. This has the effect of ameliorating the extent of protein precipitation measured. Thus the apparent depletion of CLU from the pool of soluble proteins in stressed plasma in the current study Fig. If it had been possible to elute proteins from the anti-CLU columns using nondenaturing conditions instead of the 2 m GdnHCl used in this study , the difference in mass profile for the two samples might have been even greater. The complexes in the current study were purified from stressed human plasma by immunoaffinity chromatography, which unavoidably involved eluting bound complexes with denaturing conditions. The harsh elution conditions are likely to have led to partial disruption of the complexes, thus making measurements of stoichiometry rather meaningless. For this reason we did not measure the apparent stoichiometry of the complexes in the current study. However, other bands were detected as uniquely present or more abundant in samples prepared from stressed plasma; these bands were regarded as corresponding to putative endogenous plasma client proteins for the chaperone action of CLU. It is expected that in the future, by applying a protein separation technique with greater resolution such as two-dimensional SDS-PAGE, further putative client proteins will be identified. Previous studies indicate that the chaperone action of CLU is promiscuous 6 , 9 , â€” , 14 , 35 ; thus it is likely that CLU will interact with most misfolding proteins, regardless of their identity, and that endogenous CLU-client complexes will contain a heterogeneous mix of client proteins. However, using the approaches described, identification of specific plasma proteins as clients for CLU will depend on their individual relative abundances and stabilities. Western blot analyses showed that these three putative client proteins were preferentially detected in protein fractions prepared by anti-CLU immunoaffinity chromatography of stressed plasma versus control plasma Fig. The small amounts of the putative client proteins co-purifying with CLU from control plasma Fig. However, low level nonspecific binding of client proteins to the anti-CLU immunoaffinity columns may also contribute in this regard, particularly in the case of HSA, which is known to bind to many surfaces. Many examples of such interactions are known; for example, lactoferrin 36 , protein C 37 , and myeloperoxidase 38 interact with CERU; at least 11 proteins are known to interact with FGN including vitronectin 39 , histidine-rich glycoprotein 40 , and apolipoprotein a 41 ; and over 60 different proteins are believed to interact with HSA Self-aggregation, which has been reported for both FGN 43 and HSA 44 , may also account for the detection of these proteins as larger species. This observation is consistent with CLU forming large HMW complexes with client proteins in stressed plasma, as has been described for purified proteins in buffered solutions 9 , 10 , 12 , The observation that CLU is found associated with insoluble protein aggregates in all protein deposition diseases in which this has been examined 45 suggests that CLU i binds to unfolding proteins in vivo and ii becomes incorporated into protein deposits when its chaperone action is overwhelmed by an excess of misfolded protein. All three CLU client proteins identified in this study are involved in protein deposition diseases. Co-localization of CLU with drusen proteins is very common in ARMD 15 ; furthermore, in this same disease, non-drusen FGN deposition is implicated in the atrophy of the retinal pigment epithelium and choroidal neovascularization 52 , The progression of both atrophic and neovascular ARMD is supported by platelet activation, which results in secretion of growth factors and monocyte chemoattractants. Platelet activation is enhanced by unfolding of FGN 54 and results in the release of CLU from the platelets by degranulation The effects of the interaction between CLU and stressed FGN on platelet activation are currently unknown; this may have significance not only to ARMD but also to the many ischemic and atherosclerotic vascular conditions where FGN deposition is known to occur 5 , 56 , â€” , As in ARMD, FGN deposition in such diseases is associated with the recruitment and activation of platelets and has been proposed as a mechanism for vascular injury Deposition of FGN has been reported in breast cancer 60 , mesothelioma 61 , colon cancer 62 , and lymphoma Although the reasons for this are unknown, one potential implication of this observation is that FGN deposition promotes angiogenesis and cancer progression. FGN deposits are also found in renal disease 64 , 65 , hereditary renal amyloidosis 66 , and systemic lupus erythematosus In Type 1 insulin-dependent diabetes mellitus, extracellular deposition of HSA is observed around dermal capillaries 68 , kidney 69 , 70 , skeletal muscle 71 , and the thyroid gland Given the large number of diseases in which extracellular protein deposition occurs 1 , â€” , 5 , 8 , characterization of mechanisms that clear damaged proteins in healthy individuals is likely to shed light on how protein deposition pathologies arise. In this study we have identified three plasma client proteins that bind to CLU during physiologically relevant stress. Their deposition in numerous conditions suggests that overwhelming or disruption of normal activities that prevent their accumulation in healthy individuals is important in the progression of disease. The in vivo interaction of CLU with these client proteins and others in vivo is likely to be an important mechanism to prevent the pathological deposition of misfolded extracellular proteins. Significantly, it has been shown that CLU knock-out mice develop progressive glomerulopathy, which is characterized by the accumulation of insoluble protein deposits in the kidneys This directly implicates CLU in the clearance of potentially pathological aggregating proteins, although the precise mechanism underlying this has yet to be described. It has been proposed to occur via the receptor-mediated endocytosis and subsequent lysosomal degradation of extracellular chaperone-client protein complexes 6. Evidence is rapidly accumulating that CLU and other abundant extracellular chaperones are key elements in a quality control system for extracellular protein folding 6 , â€” , 14 , 21 , 22 , 35 , 45 , 74 , â€” , This report is an important step toward a more complete understanding of the vital mechanisms involved in extracellular protein folding quality control.

Chapter 5 : Isolation of mitochondria from plant cell culture | A H Millar - racedaydvl.com

Jenkins RE(1), Kitteringham NR, Greenough C, Park BK. Author information: (1)University of Liverpool, Liverpool, Great Britain. Plasma and serum have been the focus of intense study in recent years in the expectation that they will provide important biomarkers of health and disease, without the need.

Hunter Caprion Pharmaceuticals, Inc. Immunoaffinity IA depletion is currently the most specific method for performing this step. Historically, IA depletion matrices have been designed to be used with inorganic buffers. However, the presence of salts in depleted samples presents a particular problem, and these must be removed in order to make samples compatible with post-depletion processing. Desalting dialysis, ultrafiltration, size-exclusion, etc. Moreover, these steps require additional labor, increasing the processing time and cost of analysis. In order to avoid these problems, we have developed an IA method using a volatile buffer that can be removed from depleted samples by lyophilization. This method allows the execution of reproducible and efficient depletion of blood plasma in a semi-automated manner. Immunoaffinity depletion; Volatile buffer; Plasma profiling; Proteomics; Multiple affinity removal system; Albumin; Immunoglobulin G 1. Components of these binding buffers inorganic ions have limited compatibility with downstream steps of the sample The protein profiling of blood serum and plasma is chal- processing enzymatic digestion, ion-exchange chromatogra- lenging due to the wide dynamic range of protein abun- phy, etc. Therefore, they should be removed prior to further dance that complicates the proteomic analysis. Only one study tration of low-abundance proteins and therefore improving the describing depletion using a volatile binding buffer has been detection sensitivity [2,3]. In this case Cibacron Blue and protein G were Several depletion methods for specific removal of high abun- used for the removal of albumin and IgG from cerebral spinal dance proteins have been developed. These include dye-ligands, fluid. Therefore, one of goals of this study was to compare the such as Cibacron blue F3GA [4], mimetic ligands [5,6], pro- efficiency of immuno-affinity depletion under volatile and non- teins A and G [7â€"9] or antibodies IA depletion [2,10,11]. Major manufacturers oped by Agilent Technologies was used in this study. This of IA matrices Agilent, GE Healthcare, and GenWay usu- system enables simultaneous removal of albumin, IgG, alfa ally provide depletion protocols based on non-volatile binding antitrypsin, IgA, transferrin and haptoglobin from human blood plasma. B 41â€"46 depletion MAD column can be used for multiple up to 2. Demonstrating that depletion in a volatile buffer attains the same level of reproducibility from 2. Sample preparation run to run will allow sequential unattended depletion of a Forty microliters of a thawed human plasma aliquot were large number of samples. Therefore, A for the depletion in non-volatile buffer. Two samples were another goal of this study was to determine whether depletion prepared for the depletion under volatile conditions, and one under volatile conditions can be carried out reproducibly over a sample was prepared for the depletion under non-volatile con- period of several days. Enzyme-linked immunosorbent assays ditions. Diluted samples were briefly vortexed and then spun ELISA in addition to other analytical methods were used to in a microcentrifuge. All samples were prepared individually in evaluate the method. Unbound proteins depleted were obtained from Agilent Technologies, Inc. Palo Alto, CA, plasma were collected. Further, at 11 min, the gradient was USA. Ammonium bicarbonate and Gibb- lyzed and freeze-dried. Bicinchoninic acid base protein assay BCA reagents nium bicarbonate pH 7. BCA analysis was executed Unbound proteins depleted plasma were collected. Human and the column was washed with Agilent buffer A for 4. Other bicarbonate for 5. Unless otherwise specified, all solvents and chemical reagents were of analytical grade. Freeze-drying Samples depleted using volatile buffer and raw plasma sam- 2. Lyophilized samples were A human MAD column 4. Reproducibility of the depletion using volatile buffer Sweden. Before analysis, one frozen aliquot of ods and obtain reproducible UV profiles. Samples were stored on ice and injected blood plasma. This loading amount ensures maximum removal over a period of approximately 5 h with time intervals of approx- of target proteins. As observed from the Unbound proteins depleted plasma were collected and freeze- ELISA results

Table 1 , using either volatile or non-volatile dried twice. Enzyme-linked immunosorbent assay To evaluate the losses caused by dialysis, samples depleted under volatile conditions were processed using both freeze-ELISA was performed on duplicates of each analyzed sample drying and dialysis. In Table 1, it can be seen that the sample following standard protocols with some modifications. After incubation, washing and block-tions. This suggests that losses are incurred during the dialysis. After 4 min incubation, color development was mon- the peak shape of depleted plasma proteins Fig. However, itored at nm using the PowerWave plate reader. Standard it increases the chromatography time by approximately 2 min curves were constructed from readings of eight duplicated serial compared to the original methodology of Agilent Technologies. This is due to the incompatibility of ammonium bicarbonate Commercial proteins were used to build standard curves for each buffer with buffer B of Agilent Technologies mixing of these protein of interest. Therefore, a plug of Agilent buffer A was always flowed between the ammo- 3. Results and discussion nium bicarbonate buffer and Agilent buffer B. B 41â€"46 average value. Protein patterns were similar to those of previously published results [10]. Peak heights of unbound fractions were more reproducible from run to run than from day to day. Relatively reproducible solid line and non-volatile dotted line conditions. Bound proteins elute slightly later under volatile conditions. A negative trend of the depletion effi- ciency toward IgG from It could not be Reproducibility of the depletion under volatile conditions determined whether this was related to the leakage of antibod- Day Unbound proteins Bound proteins ies from the column or to a decrease in IgG depletion effi-ciency. This may be explained 4 8 0. The sam- 5 9 0. These include the b Calculations based on per day average values of peak heights at nm. The latter was one of The run-to-run and day-to-day reproducibility of the depletion our greatest concerns, because blood plasma contains a pool of plasma injections was monitored by UV absorbance at of very active proteases that can degrade a significant propor- nm. The CV corresponds to the S. Plasma samples identical for each run loaded onto depletion column had the following protein content: Protein amounts in raw plasma and unbound fractions depleted plasma were calculated with respect to the volume of raw plasma samples loaded onto depletion column. Eliminating the sample desalting step decreases the overall sample processing time. In addition, lyophilizing the sample rather than performing dialysis minimizes sample loss. Although the MARS column has been presented here, immunoaffinity depletion in ammonium bicarbonate buffer could be implemented with other affinity matrices. This buffer system has been successfully applied in our laboratory for the depletion of blood plasma proteins with resin-coupled protein- G, A and L. Depletion under volatile conditions should therefore be generally applicable to the preparation of samples for plasma profiling using mass spectrometry. Acknowledgement We would like to thank Agilent Technologies for the kind Fig. Aliquots of raw plasma were diluted four-fold by buffer A without lines 1, 4 and 7 protease inhibitors and by buffer References A contained either CompleteTM mixture from Roche lines 2, 5 and 8 , or home- made mixture of different protease inhibitors lines 3, 6 and 9. All samples [1] N. Proteomics 1 Corzett, 4â€"6 and 16 h 7â€"9 of incubation. Neither a degradation of bands above 10 kDa, B. Steiner, Proteomics 3 The column stability is comparable using either volatile [4] E. Furthermore, the repro- [5] S. Con- ducibility of the depletion in the volatile buffer system suggests ley, Z. Therefore, the reproducibility, depletion efficiency and Prog. Jakob, meet the criteria that determine the successful implementa- P. Langen, Amino Acids 27 Pennington, Proteomics 10 Immunoaffinity depletion of proteins from blood plasma [11] D. The major [12] K. Davidsson, Proteomics 5 B 41â€"46 [13] H. Hefta, JALA 9 Junker, Protein Express Purif.

Chapter 6 : Internet Scientific Publications

In studies of the plasma proteome, the high abundance of proteins such as albumin and immunoglobulin impedes the investigation of lower abundance proteins that may be more suitable as biomarkers.

Chapter 7 : 2D PAGE : sample preparation and fractionation in SearchWorks catalog

Publications by authors named "Rosalind E Jenkins" and immunoglobulin depletion of human plasma. depletion of albumin and immunoglobulin from human plasma.

Chapter 8 : Quality Control of Serum Albumin Depletion for Proteomic Analysis | Clinical Chemistry

[et al.] -- Multi-component immunoaffinity subtraction and reversed-phase chromatography of human serum / James Martosella and Nina Zolotarjova -- Immunoaffinity fractionation of plasma proteins by chicken IgY antibodies / Lei Huang and Xiangming Fang -- Proteomics of cerebrospinal fluid methods for sample processing / John E. Hale.

Chapter 9 : Protein Depletion for Plasma and Serum Proteomic Analysis (Human plasma and s)

The low-molecular weight fraction (LMF) of the human plasma proteome is an invaluable source of biological information, especially in the context of identifying plasma-based biomarkers of disease.