Serum Proteome Changes Following HIV Infection

by

STUDENT: DAVID RICHARD HAARBURGER
STUDENT NUMBER: HRBDAV002

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Supervisor: Prof T S Pillay
Division of Chemical Pathology
University of Cape Town

Declaration

I, David Richard Haarburger, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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List of Abbreviations

2DE	Two--Dimensional gel electrophoresis
2D-Gel	Two-dimensional gel
A1AG	Alpha-1-acid glycoprotein
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine transaminase
ANOVA	Analysis of variance
ApoAI	Apolipoprotein A-1
ASMP	Abnormal spindle-like microcephaly-associated protein
CD4	Cluster designation 4
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CRP	C-Reactive protein
CSF	Cerebrospinal fluid
DTT	Dithiothreitol
ESI	Electrospray ionization
HAD	HIV-associated dementia
HAND	HIV-associated neurocognitive disorder
HIV	Human immunodeficiency virus
IEF	Isoelectric focusing
IgG	Immunoglobulin G
IPG	Immobilised pH gradient
LC	Liquid-chromatography
MALDI	Matrix-assisted laser desorption ionization
MMP9	Matrix metalloproteinase 9
MS	Mass spectrometer/Mass spectrometry
MSDB	Mass Spectrometry protein sequence database
NCBI	National Centre for Biotechnology Information
SDS	Sodium dodecyl sulfate
T2CK1	Type II cytoskeletal keratin 1
TCEP	Triscarboxyethyl phosphine
TFA	Trifluoroacetic acid

TOF	Time of flight
Tris	Trishydroxymethylaminomethane

PART A

Protocol

ORIGINAL PROTOCOL AS APPROVED BY THE DEPARTMENTAL RESEARCH COMMITTEE AND FACULTY RESEARCH ETHICS COMMITTEE

Principal Investigator

Academic Pathology Department: Chemical Pathology

Principal Investigator

Tahir Pillay: Chief Specialist, Professor & Head, Division of Chemical Pathology

Groote Schuur and Red Cross Children's Hospitals

University of Cape Town

MP 0275905

profts.pillay@uct.ac.za

Tel: 021 406 6185

Project Leader

David Haarburger: Registrar

National Health Laboratory Service

Division of Chemical Pathology

University of Cape Town and Groote Schuur Hospital

MP 0576549

david.haarburger@uct.ac.za

Tel: 021 404 4135

Degree registered:	MMed(Chemical Pathology)
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Project Title

Biomarker discovery in HIV/AIDS using proteomics.

Short Description of the Project

The objective of this project is to use proteomics to identify differentially expressed proteins in serum from patients with HIV in the hope of detecting novel biomarkers for HIV/AIDS and associated complications. Samples will be obtained from HIV clinics and vaccine centres associated with the Desmond Tutu HIV Centre, UCT and analyzed using proteomic techniques in collaboration with the Swegene Proteomics Centre. The proteome of HIV positive serum will be compared with the proteome of HIV negative serum. Biomarkers identified in this way will be characterised further for specificity using immunoassays.

Collaborators

Dr Jörgen Bergström
Swegene Proteomics Centre.

University of Goteborg
Lund, Sweden

Linda Gail-Bekker
Desmond Tutu HIV Centre
Institute of Infectious Disease & Molecular Medicine
University of Cape Town

Project Details

Aim and objectives

The specific objectives of this project are to:

a) Establish a base of proteomics expertise as it applies to diagnostic clinical chemistry, in the Division of Chemical Pathology at the University of Cape Town and GSH NHLS laboratory and in the long-term within the broader context of the greater NHLS.
b) Use proteomics as a basis to identify new biomarkers in HIV infection and eventually in other diseases of national priority;
c) Create a link between basic and applied research;
d) Make a meaningful contribution to research capacity development;
e) Foster long-term links with other institutions in the NHLS umbrella;
f) Use this information from this project to develop and commercialize new immunoassays under the NHLS umbrella.

Background

The burden of HIV/AIDS on health services and economies worldwide and especially in Africa cannot be overstated. Currently, the diagnosis of HIV is dependent on the detection of viral protein or antiviral antibody in human serum [1, 2] or more recently, the detection of viral nucleic acid in serum. CD4+ cells and viral load can also be used as a marker of disease progress and to monitor response to

antiretroviral therapy.[1, 2] It will be useful to be able to identify protein markers that are induced during HIV infection and can be used to diagnose and monitor disease progression and the complications of therapy.

Proteomics is thus the study of the large-scale expression, function, and interaction of the complement of proteins in an organism in health and disease.[3] Recent advances in proteomic technologies permit the evaluation of systematic changes in protein expression in response to intrinsic or extrinsic perturbations to the biologic system, for example, those that occur in infectious diseases. Proteomics is a potential tool for the discovery and application of novel biomarkers in diagnosis of the inception and progression of diseases, which might then affect prevention and therapy. Serum proteome analysis has the potential to facilitate disease diagnosis and monitoring.[4] Previous studies have shown that this is a formidable approach to identify new biomarkers, especially in the field of cancer research.

Proteomics has emerged as a relatively new field of protein science based on a "classical" electrophoretic technique and is now dominated by separation methods including traditional 2-D electrophoresis and liquid chromatography to separate proteins, and methods to analyse proteins by mass spectrometry.[5] Coupled with bioinformatics and rapidly evolving software, these techniques have become powerful methods for protein characterization and identification. In the diagnostic arena, these techniques have been used to characterize protein profiles from normal and diseased body fluids.[4, 6] Blood has direct contact with almost all of the tissues in the human body and therefore pathological changes are likely to be reflected by proteomic changes in serum. Biomarkers identified in serum may form the basis for simple, non-invasive diagnostic or monitoring tests. There have been a number of developments in proteomics which have enhanced its utility beyond the research lab. Proteomic analysis is being applied to the identification of biomarkers in a number of other disease states.[7-9] Proteomic analysis of human serum for identification of disease-specific biomarkers promises to be a powerful diagnostic tool for defining the onset, progression and prognosis of human diseases.[4, 10-12] Serum provides an abundant sample for diagnostic analyses because of the expression and release of proteins (potential biomarkers) into the bloodstream in response to specific physiological states such as viral infections, bacterial infections, cancer and

Alzheimer's disease to name a few. Therefore, serum offers a medium to define differential expression characteristics specific to those physiological states.

The aim of this study is to use proteomics to identify novel proteins that are induced in the human serum proteome following HIV infection and the development of AIDS.

Detailed methodology

This project has been approved by the UCT Research Ethics Comitee (Rec Ref 211/2007). The project will be conducted between August 2007 and June 2009. Volunteers will be selected from patients visiting the HIV clinics and vaccine centres associated with the Desmond Tutu HIV Centre. Serum will be obtained by venepuncture from 30 HIV positive and 30 HIV negative Xhosa men aged between twenty-one and thirty-five. All volunteers will not be on any medication and will not have a history of any systemic illness. A creatinine, alanine transaminase, C-reactive protein and a random glucose will be done on all samples to rule out disease. All of these samples will be suitably anonymised. Albumin and immunoglobulins make up more than 70% of the proteins in human serum. Albumin and IgG will be depleted from the serum using the ProteoPrep Blue Albumin depletion kit (Sigma Chemical Company). The depleted sample will then be subjected to isoelectric focussing and SDS gel electrophoresis using the Ettan IPGphor and Ettan DALT II (Amersham Biosciences/GE Healthcare). The gels will then be stained with fluorescent stains such as Sypro Ruby. Alternative the proteins can be fluorescently labelled with CyDyes. The gels will be scanned and analysed to identify differentially abundant protein spots using relevant software (eg ImageQuant software by GE Healthcare). The statistical significance of differences in the intensity of protein spots will be determined using t-tests on the gels in each group. Protein spots with a relative ratio of > 1,5 and a t-test value <0,05 will be considered significant.

Differentially expressed protein spots will be subjected to robotic in-gel digestion (Ettan Spot Handling Workstation) using trypsin after reduction with DTT and alkylation with iodoacetamide. A portion of the resulting digest supernatant will be used for matrix assisted laser ionization desorption mass spectrometry (MALDI-MS)

analysis. Spotting will be performed robotically (ProMS) with ZipTips and peptides will be eluted from the C18 material with matrix (R-cyano 4-hydroxy cinnamic acid) in 60% acetonitrile, 0.2% TFA. MALDI-MS data will be acquired on an Applied Biosystems Voyager DE-STR instrument and the observed m/z values will be submitted to ProFound for peptide mass fingerprint searching using the NCBI nonredundant database. Those samples that proved inconclusive following MALDI-MS will be analyzed by LC/MS/MS on a Micromass Q-Tof Ultima. The MS/MS data will be used for database search using MASCOT software (Matrix Science).

All of the mass spectrometric analysis will be carried out in Sweden at the University of Goteborg in collaboration with Dr Jörgen Bergström. The samples will be collected and prepared in Cape Town.

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Envisaged outputs/outcomes

It is envisaged that this study will elucidate some of the proteomic changes associated with HIV and help identify possible target proteins which will provide the basis for developing immunoassays to monitor the severity of HIV infection and monitor the complications. The work will be presented at the South African Society of Pathologists annual meeting, the annual conference of the Association for Clinical Biochemistry (UK) and the American Association of Clinical Chemistry (USA). The study will provide material for Dr Haarburger's MMed dissertation and will provide Dr Haarburger with the opportunity to publish in a major peer-reviewed journal such as Journal of Clinical Pathology or Clinical Chemistry.

Impact

Dr Haarburger will be the project leader. He is currently training as a Registrar in Chemical Pathology and is registered for the MMed Degree at UCT. The project will establish a base of proteomics expertise as it applies to diagnostic clinical chemistry, in the Division of Chemical Pathology at the University of Cape Town. The project will form an adjunct for collaboration with the University of Gotheborg in Sweden and will therefore be important for enhancing institutional capacity. Furthermore,

the project will develop capacity in a new and emerging area that is under-researched in the South African context.

Institutional Approval

This proposal has been approved by the University of Cape Town Research Ethics Committee.

Funding

Funding is provided by the National Health Laboratories Research Trust

Amendments to Original Protocol

1 **Title** – The project title was changed from *Biomarker discovery in HIV/AIDS using proteomics* to *Serum proteome changes following HIV infection* to more accurately reflect the research.

2 **Gel staining** – Due to the unavailability and high cost of fluorescent scanners, fluorescent dyes could not be used. Silver staining was used as an alternative.

3 **Mass spectrometric analysis** – Due to the difficulties in transporting biological samples, samples were analysed at the Centre for Proteomic and Genomic Research, Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town and not at the Swegene Proteomics Centre, University of Goteborg, Lund, Sweden.

PART B

Biomarker discovery in HIV using proteomics

Introduction

Proteomics involves the analysis of the full array of proteins produced by an organism. Over the last two decades, new techniques have revolutionised the task of determining a proteomic profile. Since the Human Immunodeficiency Virus (HIV) pandemic currently affects almost 40 million people, and has been responsible for the death of over 25 million, it is logical to ask what role proteomics can play in understanding and overcoming this disease. The intent of this review is to assess the current state of proteomic techniques and its application to HIV research. Some of the contributions of proteomics to our current understanding of the pathogenesis of HIV infection will be highlighted, as well as the role of biomarkers in disease and their potential role in monitoring HIV.

Proteomics

Proteomics can be defined as the study of the full set of proteins expressed by an organism, tissue or cell, and the change in their expression patterns under different conditions. Proteomic techniques initially developed in the 1990's from analytical biochemical techniques used for protein separation and have evolved to provide the researcher with a set of new tools to help understand biological processes at the molecular level. Although great strides have been made since then with the currently available genomic toolbox and associated bioinformatic tools, these techniques had several limitations.[1] Understanding the biology of cells at the genetic level is not always equivalent to understanding it at the protein level, and this is crucial since it is the proteins that are the functional molecules of the cell.

Proteomics has been used to identify proteins involved in pathological processes and to evaluate changes in protein expression during illness. A major focus of clinical proteomics has been cancer research but the techniques are equally valid when studying infectious diseases. Proteomic methods can be used in various ways to study infectious diseases. For example, the pathogen itself may be studied, the host or immune response to the pathogen can be examined, or the mechanism of action of antimicrobials can be determined. A vast amount of research has also been applied to the development of biomarkers for the diagnosis of diseases and for the monitoring of their progress.

Proteomic Techniques

Proteomics uses a combination of several techniques including electrophoresis, image analyses, mass spectrometry (MS), amino acid sequencing and bio-informatics to comprehensively resolve, quantify and characterise proteins.[2] The main steps involve protein separation, quantification, if relevant, and protein identification.

Protein separation

For analysis of proteomes, protein separation has traditionally been performed by 2-dimensional gel electrophoresis (2DE). This technique was first described in 1975,[3, 4] and dominated proteomics for the following 25 years. It involves a primary separation based on the isoelectric point of individual proteins called isoelectric focusing, followed by a secondary separation based on protein size using polyacrylamide gels. 2DE has many advantages. It is an efficient and versatile method and has been used for the separation of complex protein mixtures.[5] It produces high-resolution separation and allows for protein quantification both within a sample and between samples.[6] It is reproducible and sensitive, and under the appropriate conditions has been able to resolve over 10 000 proteins.[7]

However, 2DE has its limitations. Despite its impressive resolution, it is still not adequate considering the enormous diversity of cellular proteins, and comigrating proteins in the same spot are not uncommon.[8] Thus, zoom or narrow pH range gels are required to increase the resolution and to greatly decrease the probability of this

problem.[9] Some proteins, such as membrane and nuclear proteins, are poorly water-soluble and tend to precipitate during isoelectric focusing. Recent advances have improved the situation, but it appears that this will remain a major limitation of 2DE.[6]

Proteins in human tissues have a huge dynamic range. In serum for instance, albumin is present at 40 g/L and cytokines at ng/L levels (10^9 dynamic range). This is clearly out of the range of 2DE which has a maximum dynamic range of 10^4.[6] Proteins are heterogeneous and cover a wide range of isoelectric points and masses. No single gel can routinely cover all the proteins. These problems, of course, are not unique to 2DE and will complicate all separation techniques. What is unique to 2DE is the fact that it is costly, labour-intensive and not easily automated.[10]

In an attempt to overcome some of these limitations, alternative protein separation methods have been developed. These include: one-dimensional gel electrophoresis followed by mass spectroscopy analysis;[6] electrophoresis-free, liquid chromatography (LC)-based approaches such as multi-dimensional protein identification technology (MUDPIT);[11] or isotope-coded affinity tags (ICAT).[12] Each of these methods has its own advantages and disadvantages.

Protein visualisation

Several stains can be used to visualise proteins after 2DE, each with its own benefits and drawbacks. The more commonly used stains are Coomassie brilliant blue (detection limit 8-18 ng) and silver staining (detection limit 2-4 ng).[2] In addition, fluorescent dyes can be used (detection limit 1-2 ng) which show superior sensitivity and linearity.[13] The use of different fluorescent dyes allows multiple samples to be pooled and run on the same gel. This development is called difference gel electrophoresis[14] and is more reproducible and accurate than traditional 2DE, which requires two gel plates.[15]

Protein identification

Early protein analysis consisted of identifying proteins separated by gels. Purified proteins would be sequenced (either intact or after enzymatic digestion) from the amino terminus using Edman degradation. Systematic sequencing programs combined with information from gene sequencing led to the rapid increase in the size of sequence databases, and the chance that a particular protein sequence was already represented in a protein or gene sequence database also increased. Thus the complete sequence of a protein of interest could be found by database searching, without the need for its sequence to be determined *de novo*. This was the beginning of discovery proteomics.

Although protein sequencing by Edman degradation was reliable and automated, it was also slow and had a poor sensitivity,[10] so better protein identification techniques were needed. At that time, mass spectrometry had been used for analysing small molecules. However, peptides and proteins, like other large molecules, proved difficult to ionize under conditions that did not destroy the molecule, and thus were unsuitable for mass spectrometry. In the late 1980s, two methods were developed that overcame this obstacle and allowed the ionization of peptides and proteins at high sensitivity without causing excessive fragmentation. These breakthroughs were electrospray ionization (ESI)[16] and matrix-assisted laser desorption ionization (MALDI).[17] The success of these ionization methods in analytical protein chemistry led to the development of commercial mass spectrometers equipped with robust ESI or MALDI 'ion source' instruments, which rapidly penetrated the protein chemistry community.

Although MALDI mass spectrometers can determine the mass of a protein or peptide with a high degree of accuracy, the intrinsic mass of a protein is not a unique identifying feature. It was quickly recognized, however, that the masses of the various peptides generated by fragmentation of an isolated protein with an enzyme of known cleavage specificity could uniquely identify a protein. Implementation of this discovery in database search algorithms, together with mass spectrometry peptide analysis, was used to identify proteins and is called peptide mass fingerprinting.[18-22]

In addition to measuring peptide mass, some of these instruments can isolate specific ions from a mixture on the basis of their mass-to-charge (*m/z*) ratio and fragment these ions in the gas phase within the instrument, allowing the recording of MS/MS spectra. This fragmentation fingerprint is compared with the theoretical masses of all the fragments, which have been obtained from a selected protein database. This is called peptide fragment fingerprinting.[23]

Because peptide ions fragment in a sequence-dependent manner, the MS/MS spectrum of a peptide, in principle, represents its amino acid sequence. Thus, a sequence of a few amino acids can be obtained from an MS/MS spectrum. This amino acid sequence can be used in a classical database search to identify the protein.

Biomarkers

Biomarkers are not new. Laboratory tests, x-rays, electrocardiograms, and other established procedures have long been used to demonstrate the presence of disease and response to treatment. Biomarkers may be used to measure disease predisposition, to diagnose or stage a disease or to determine its severity, prognosis, or outcome.[24] Many are familiar to clinicians and have been used for some years now. For example, carcinoembryonic antigen and CA-125 are used to monitor cancer,[25] haemoglobin A_{1c} is used for monitoring diabetes,[26] and rheumatoid factor is used to diagnose and provide a prognosis in rheumatoid arthritis.[27] New tools are now being applied to biomarker discovery that have developed out of technological advances and the growing understanding of molecular biology. These tools come from the fields of genomics, epigenomics, transcriptomics, proteomics, lipidomics, and metabolomics.

Biomarkers can be identified using proteomics as follows: plasma or tissue is obtained from two populations, one containing the investigated disease and the other a suitable control group. The proteins in each sample (the entire proteome) are separated (by a technique such as 2DE) and identified by mass spectrometry. Differences in the proteins in the control and diseased populations are sought using a

variety of techniques. Proteins uniquely present in the diseased tissue or condition can serve as a diagnostic or prognostic biomarker.

However, there have been many challenges in translating plasma proteomics from bench to bedside and relatively few plasma biomarkers have successfully made the transition from proteomic discovery to routine clinical use.[24] Obstacles to this translation include the complexity of the proteome in biologic samples, the presence of highly abundant proteins such as albumin in biologic samples that hinder detection of less abundant proteins, false-positive associations that occur with analysis of high dimensional datasets, and the limited understanding of the effects of growth, development, and age on the normal plasma proteome.[28]

HIV

The HIV pandemic has resulted in the death of over 25 million people and the virus currently infects almost 40 million of the world's population,[29] and Sub-Saharan Africa especially South Africa carries the greatest burden of the HIV epidemic. Although tremendous progress has been made over the years, understanding of the pathogenesis and treatment of the infection is still not optimal.

Currently, quantification of viral RNA levels (viral load) and cluster designation 4 (CD4) cell count determinations represent the most widely used laboratory assays employed in HIV patient management. Viral load is used by the clinician to measure the baseline disease burden prior to initiation of antiretroviral therapy, to assess the efficacy of antiviral drug treatment, to detect the onset of drug resistance, to predict the development of acquired immunodeficiency syndrome (AIDS) -related opportunistic infections and to monitor disease progression.[30] Clinical data also suggest that the viral RNA level at the initial phase of HIV infection (known as the set-point) can be used as a prognostic marker for HIV disease progression.[31] CD4 counts are used to decide when to initiate therapy, to monitor the response to therapy, and to assess the risk of developing opportunistic infections.[32] CD4 counts are additionally helpful to evaluate possible treatment failure and to monitor disease progression.[33]

However, these tests are not possible in all settings, particularly where resources are limited. They can be prohibitively expensive, are complex to perform, and require advanced laboratory infrastructure and highly skilled laboratory technicians.[34, 35] However, inadequate monitoring of HIV progression in adults taking antiviral treatment is associated with detrimental outcomes.[34, 36, 37] For these reasons, alternative ways to monitor HIV have been sought. These include various serologic HIV-1 p24 antigen detection methods,[38, 39] a real-time immuno-polymerase chain reaction assay that uses HIV-1 p24 antigen as a marker for quantification of viraemia,[40] qualitative and quantitative cell culture methods, and cheaper methods of enumerating CD4 cell numbers.[35] The question arises whether there is a role for proteomics in increasing our understanding of HIV or in the management of HIV-infected patients. Conceivably, it may lead to a superior method of monitoring HIV.

HIV Proteomic Research

Proteomic methods can be applied in various ways to study HIV. The proteome of the virus itself can be analysed. Cells infected with HIV can be compared to normal cells to determine the effect on intracellular proteins. The cell culture medium of cultured HIV-infected cells can be analysed (the secreteome), or plasma can be used to assess whole body changes to HIV infection. The various proteomes can be monitored for changes in response to infection or to changes related to treatment. The remainder of this review will examine the HIV research performed using proteomic methods. In order to illustrate the diverse uses of proteomics, it is grouped according to sample type. The proteome of HIV is continuously being studied and comprehensive, up-to-date databases are maintained by the Los Alamos National Laboratories (www.hiv.lanl.gov) and by BioAfrica (www.bioafrica.net).

Virions

Proteomics has been useful in determining the protein structure of HIV virions, particularly in determining post-translational modifications and in identifying host proteins that become incorporated into the virion – both of which are not obvious from genomic information. 2DE and MALDI time-of-flight MS were used to analyse the lysate of purified HIV virions.[41] Twenty-five proteins inside the virion

including a N-terminal formylated isoform of $p24^{gag}$ were identified. In another study[42] LC-MS/MS was used to analyse host proteins in the HIV virion, and CD48 and histones (specifically histones H1, H2, H3, and H4) were discovered to be incorporated in the virion.

Macrophage secretions

HIV-infected and -uninfected macrophages have been cultured and the proteome of the media analysed.[43] It was shown that matrix metalloproteinase 9 (MMP9) was down-regulated in HIV-infected cells and also that this decrease was greatest in cells with higher reverse transcriptase activity. This is significant because MMP9 is a well known neurotoxin as shown in cerebrovascular injury, seizures and Alzheimer's disease.[44] In a subsequent study,[45] six differentially expressed proteins were found. These included: cystatin B, cystatin C, L-plastin, leukotrine A4 hydrolase, α-enolase, and chitinase 3-like 1 protein (HC-gp39). The authors noted that these differentially expressed proteins represented a wide range of functional groups and include structural (cytoskeleton) proteins, proteins involved in redox reactions, regulatory proteins and enzymes. They also reported that following HIV infection, the macrophage is stimulated and the number of secreted proteins increased. This observation supports the notion that the virus accelerates production and secretion of macrophage proteins to its advantage.

Cerebrospinal fluid

Approximately half of HIV-infected individuals develop some form of HIV-associated neurocognitive disorder (HAND), which is comprised of HIV-associated dementia (HAD), mild neurocognitive disorder, and asymptomatic neurocognitive impairment.[46] Currently, the diagnosis of HAND is a diagnosis of exclusion made primarily on clinical grounds requiring the elimination of concurrent opportunistic infections, psychiatric disorders and malignancies. In order to aid with diagnosis, much has been expended to find a biomarker to support the diagnosis of HAND. Cerebrospinal fluid (CSF) has been analysed from HIV-positive individuals with and without HAD.[47] Twenty differentially expressed proteins were identified, six of which (vitamin D binding protein, clusterin, gelsolin, complement C3, procollagen C-endopeptidase enhancer 1 and cystatin C) were validated by Western blot

analyses. In another study [48] searching for biomarkers of HAND in CSF, nine proteins unique to HIV cognitive impairment were identified, including soluble superoxide dismutase, migration inhibitory factor - related protein 14, macrophage capping protein, neurosecretory protein VGF, galectin-7, L-plastin, acylphosphatase 1, and a tyrosine 3/tryptophan 5-monooxygenase activation protein. Further work is required in order to establish which protein or combination of proteins will make an ideal biomarker of HAND.

Serum or plasma

The plasma from subjects with the AIDS has been compared to that from healthy individuals.[49] The expression of seven proteins was found to be increased (alpha-1-antichymotrypsin, antitrypsin, ALB protein, haptoglobin beta chain, immunoglobulin light chain, haptoglobin alpha-2 chain, and transthyretin) whilst one (apolipoprotein A-I) was decreased. Furthermore, a change of expression of the various isoforms of apolipoprotein A-I was observed. The authors hypothesised that this may be used to monitor the progress of HIV infection. Serum has also been used to search for biomarkers of HAND.[50] Immunodepleted serum samples from patients with and without HAD were compared and it was discovered that gelsolin and prealbumin were differentially expressed in these patient groups.

Brain microvascular endothelial cells

The blood-brain-barrier dysfunction in HIV infection has also been investigated directly.[51] Brain microvascular endothelial cells were co-cultured with HIV-infected and -uninfected monocyte-derived macrophages. The endothelial cells were lysed and the lysate was subjected to two-dimensional difference gel electrophoresis. Structural, cytoskeletal, regulatory, metabolic, voltage-gated ion channels, heat shock, transport and calcium binding proteins were shown to be significantly upregulated in endothelial cells due to interactions with HIV- infected macrophages. These studies provided proof that HIV-infected macrophages affect the endothelial cells and can, in this way, affect blood-brain-barrier dysfunction and the development of HIV central nervous system disease.

T-cells

Since T-cells play such a crucial role in the pathogenesis of HIV, it is logical to study their proteome. In a study that focussed specifically on plasma membrane-associated proteins on a chronically HIV-infected T-cell line (ACH2), 17 differentially expressed proteins were discovered.[52] The majority of the identified proteins (65%) were integral membrane or membrane-associated proteins that could be divided into two functional categories – proteins involved with cell adhesion, structure, and migration, and receptors and receptor-associated proteins. The receptor and receptor-associated proteins were involved in the regulation of cell death and survival and included X-linked inhibitor of apoptosis. This protein was increased, and it may be that up-regulation of this anti-apoptotic protein is a mechanism to increase cell survival to counterbalance the apoptotic effects of some viral accessory proteins.

The lipid metabolism-altering effects of both HIV and HIV therapy have been well documented.[53] A T-cell line (RH9) was studied before and after HIV infection to investigate if the T-cells showed alteration in the production of any proteins involved in lipid metabolism.[54] After HIV infection, 18 proteins were differentially expressed, 12 of which were exclusively expressed in HIV-infected cells. Seven proteins belonged to various families of enzymes/kinases (complement C3 peptidase, phosphatidylinositol-4-phosphate 3-kinase C2 domain containing beta polypeptide, fatty acid synthase, glutathione peroxidase-1, protein kinase C beta, long chain-fatty-acid-CoA ligase 1, epidermal fatty acid binding protein); five were transporter proteins, two were transmembrane receptors, two were molecular chaperones and there was one each of the ligand-binding and adapter-like proteins. The low-density lipoprotein receptor 1 and the very low-density lipoprotein receptors were found to be upregulated after HIV infection. Among all the differentially regulated proteins, apolipoprotein-B100 showed the greatest increase post- HIV infection. Apolipoprotein-A-I was shown to be synthesised in HIV-infected but not in uninfected cells. It was concluded that the replication of HIV in human T-cells alone alters the synthesis of novel enzymes, kinases and other proteins that enhance fatty acid synthesis, increase lipid peroxidation (crystallization), disrupts lipid metabolism and reduces lipid clearance without any influence of genetic or epigenetic factors.

Presumably, these changes in lipid metabolism may also occur in other cell types and may potentially contribute to the dyslipidaemia observed in many patients with HIV infection.

Cervical lavage samples

A group of Kenyan sex workers has been shown to be relatively resistant to HIV.[55] It has been suggested that the mucosal layer in the cervicovaginal compartment may play a role in mediating this resistance. In order to study this further, cervical lavage specimens from Δ-32-CCR5-negative and HIV-negative (resistant) sex workers were compared to various controls.[56] Comparison of protein profiles revealed that a group of antiproteases was upregulated in HIV-1-resistant women. These included those from the serpin B family (B1, 3, 4, and 13), alpha-2 macroglobulin-like 1, and cystatin A, all of which have antiprotease or anti-inflammatory properties. It is possible that overexpression of these proteins confers protection by maintaining the integrity of the epithelial barrier.

Conclusion

Since its first discovery in the early 1980's almost 65 million people have been infected with HIV. Significant strides have been made since in our understanding of the pathogenesis and treatment of HIV infection, but currently available monitoring and treatment options are far from ideal. Since HIV monitoring has been shown to be essential when treating patients, cheaper and simpler markers of HIV infection are necessary. Proteomics has provided a new tool for researching HIV and has made some significant contributions. One of these is the discovery and identification of potential biomarkers for HAND, both in CSF and serum. Hopefully, this approach can be applied to the HIV infection itself. Ideally, it will lead to the classification of proteins involved in the progression of HIV and allow the development of new biomarkers for HIV.

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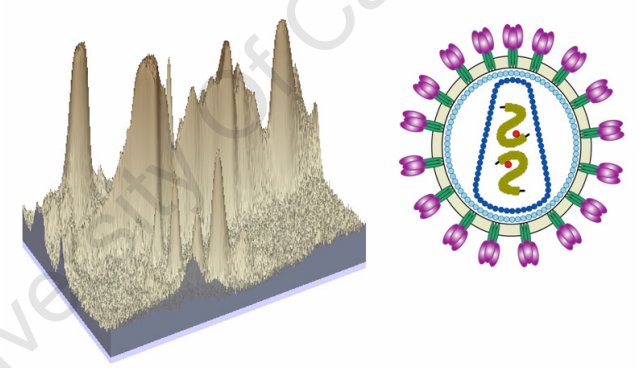
PART C

Manuscript for Submission to the *Journal of Proteome Research*

Serum Proteome Changes Following HIV Infection

Synopsis



The aim of this study was to develop additional biomarkers for human immunodeficiency viral infection. Two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry were used to compare the serum proteome of HIV-positive subjects to HIV-negative subjects. Eleven protein spots were identified as being significantly different between the two groups. Nine spots were significantly decreased and two spots were significantly increased in the HIV-positive group compared to the HIV-negative group.

Serum Proteome Changes Following HIV Infection

Author List

David Haarburger
Division of Chemical Pathology
National Health Laboratory Services
University of Cape Town

Jörgen Bergström
Proteomics Core Facility
University of Gothenburg

Tahir S Pillay
Division of Chemical Pathology
National Health Laboratory Services
University of Cape Town

Abstract

The aim of this study was to compare the serum proteome of stage I HIV-positive subjects to HIV-negative controls in order to identify novel protein changes that are induced in the human serum proteome following HIV infection. Samples were separated into HIV-positive and HIV-negative groups, and subjected to two-dimensional gel electrophoresis. The gels were silver-stained and analysed to detect significant differences between protein spots. Significant spots were digested by trypsin and subjected to analysis using MALDI-TOF mass spectrometry. Eleven spots were found to be significantly different between the two groups. Nine spots were significantly decreased and two spots were significantly increased in the HIV-positive group compared to the HIV-negative group. The proteins found to be decreased were identified as abnormal spindle-like microcephaly-associated protein,

type II cytoskeletal keratin, alpha-1-acid glycoprotein, 60S ribosomal protein L4, haptoglobin, apolipoprotein A-I, zinc finger CCCH domain-containing protein 13 and haemoglobin beta chain. The proteins found to be increased were identified as coiled-coil domain-containing protein 49 and 60S ribosomal protein L4. This information could be used to develop immunoassays to analyse protein changes in the clinical laboratory as an additional method of monitoring changes in HIV infection.

Keywords

HIV • Biomarker • Proteomics

Introduction

The Human Immunodeficiency Virus (HIV) pandemic has resulted in the death of over 25 million people and the virus currently infects almost 40 million of the world's population.[1] Although tremendous progress has been made over the years in the monitoring and treatment of HIV, the management of this disease is still not optimal.

Currently, quantification of viral RNA levels (viral load) and cluster designation 4 (CD4) cell count determinations are the most widely used laboratory assays employed in monitoring HIV patients. Viral load is used by the clinician to measure the baseline disease burden prior to initiation of antiretroviral therapy, to assess the efficacy of antiviral drug treatment, to detect the onset of drug resistance, to predict the development of acquired immunodeficiency syndrome (AIDS) -related opportunistic infections and to monitor disease progression.[2] CD4 counts are used to decide when to initiate therapy, to monitor the response to therapy, and to assess the risk of developing opportunistic infections.[3] CD4 counts are additionally helpful to evaluate possible treatment failure and to monitor disease progression.[4]

However, these tests are not possible in all settings, particularly where resources are limited. They can be prohibitively expensive, are complex to perform, and require advanced laboratory infrastructure and highly skilled laboratory technicians.[5, 6]

However, inadequate monitoring of HIV progression in individuals taking antiviral treatment is associated with detrimental outcomes.[5, 7, 8] For these reasons, alternative ways to monitor the progress of HIV have been sought.[6, 9-11]

Human plasma or serum is potentially the single most informative sample that can be obtained from an individual that describes their current state of health. It is the primary clinic specimen, easy to collect and potentially contains proteins from every tissue in the body.[12, 13] Plasma proteins have proven very useful for diagnostic, prognostic, and therapeutic development. With proteomic tools recently established, profiling of the human plasma proteome has become easier in the quest for disease-related markers.[13] Researchers have looked to serum to find potential biomarkers for hepatitis B, hepatitis C, and human T-cell leukaemia virus[14] among other diseases, and serum has been used in HIV-positive patients to find biomarkers for HIV-associated neurocognitive disorders.[15]

In view of the success of the above studies, the aim of this study was to search for biomarkers of HIV infection in serum. This was achieved by comparing the serum proteome of asymptomatic HIV-positive and HIV-negative subjects using two-dimensional gel (2D-gel) electrophoresis profiles. Proteins were identified using peptide mass fingerprinting generated by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS).

Materials and Methods

Study Population. The study was performed with ethics approval from the Research Ethics Committee of the Health Sciences Faculty of the University of Cape Town (REC REF 211/2007). All subjects were enrolled at the Masiphumelele primary health clinic. Only Xhosa males between 21 and 35 years of age were admitted to the study. All subjects who agreed to participate signed a consent form and completed a questionnaire regarding their health. The health questionnaire was used to ensure only asymptomatic subjects (World Health Organization Clinical Stage I)[16] were selected and that there were no comorbid conditions. It also ruled out any subject who was taking any form of medication which may complicate the

analysis. A copy of the health questionnaire is available in the supporting documentation.

HIV Testing. HIV testing was performed on site at the clinic. All subjects were counselled before testing. Testing was performed on a finger-prick sample using a First Response HIV Card Test (PMC Medical Pvt Ltd). If this test was positive, repeat testing was performed on a HIV-1/2 Triline Card Test (Pareekshak). Both these tests are immuno-chromatographic assays sensitive to antibodies against HIV-1 antigens (gp120, gp41 and p24) and an HIV-2 antigen (gp36).

Sample Collection and Storage. Blood was collected from the left cubital fossa of subjects into BD Vacutainer SST II Advance tubes. The blood was spun at 4000 *g* for 5 min to separate the serum. An aliquot of the serum was taken for biochemical testing. The remaining serum was frozen and stored at -70ºC until 2D-gel electrophoresis could be performed. All samples were fully processed within 6 h.

Serum Biochemistry. Creatinine, alanine transaminase (ALT), C-reactive protein (CRP) and glucose were measured immediately upon arrival at the laboratory on a Roche/Hitachi modular analyser. Samples with creatinine values greater than 120 µM, glucose greater than 7.0 mM, ALT greater than 40 U/L or CRP greater than 5.0 mg/L were excluded from further analysis.

Sample Preparation. Albumin and immunoglobulin G (IgG) were removed from 10 µL of each sample using an albumin/IgG Removal Kit (ProteoSeek). The samples were diluted and mixed with immobilised Cibacron Blue/Protein A gel slurry as directed by the package insert. 20 µL of the albumin/IgG-free filtrate was then dissolved in 130 µL of rehydration buffer (8 mM urea, 4% CHAPS, 0.0002% bromophenol blue, 50 mM dithiothreitol (DTT), 0.5% ampholyte solution).

Isoelectric Focusing. Immobilised pH gradient (IPG) strips (Bio-Rad 7cm pH 3-10 ReadyStrips) were left overnight to rehydrate in 125 µL of the sample/rehydration buffer covered with mineral oil. Isoelectric focusing (IEF) was performed at increasing voltages cumulating at 10 000 V with over 15 000 Vh of potential time. After completion of the IEF, the strips were washed for 15 min in 5 mL of equilibration buffer (50 mM Tris-HCl pH 8.8, 6 M urea, 30% glycerol, 2% sodium dodecyl sulfate (SDS), 0.0002% bromophenol blue) with 50 mg of DTT. The strips were then washed for a second time for 15 min in 5 mL of equilibration buffer with 125 mg of iodoacetamide. A final third wash was performed for 15 min using plain equilibrium buffer.

Polyacrylamide Gel Electrophoresis. A 10% polyacrylamide gel (375 mM Tris-HCl, 10% acrylamide/bisacrylamide, 0.1% SDS, 0.05% ammonium persulfate and 0.0005% tetramethylethylenediamine) was prepared. The IPG strip was placed along the top of the polyacrylamide gel and covered with 2% agarose gel. Electrophoresis took place at 60 mA for 1 h. The gels were fixed overnight in a solution containing 10% acetic acid and 40% ethanol.

Silver Staining. The gels were washed in a 30% ethanol solution for 20 min and then in distilled water for 20 min, after which they were sensitized in a 0.02% sodium thiosulphate solution for 1 min. The gels were washed again in water and then left in a silver stain solution (0.2% silver nitrate, 0.074% formaldehyde) for 20 min. A fourth wash with water was performed, after which the gels were put into developer solution (3% sodium carbonate, 1.85% formaldehyde, 0.0005% sodium thiosulfate) for 5 min. After a final wash, the process was stopped with a 0.5% glycine solution.

Gel Analysis. Gels were analysed with 2D-gel analysis software (Melanie version 7.03). The following parameters were used to detect spots on the gel: smoothness: 2, saliency: 50, min area 5. Spot differences were considered significant if the mean ratio between the HIV-positive and HIV-negative groups was greater than two or less than a half, and the p value was less than 5% as calculated using Melanie's built-in ANOVA calculator. All spots considered significant were manually checked to ensure correct matching.

Protein Extraction. Protein analysis was performed at the Centre for Proteomic and Genomic Research (Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town). Gel slices supplied were cut up into 1 mm × 1 mm × 2 mm pieces and destained in Eppendorf 1.5 mL tubes with 200 mM NH_4HCO_3:acetonitrile 50:50 (Romill; Sigma) until clear. Samples were dehydrated and desiccated before reduction with 5 mM triscarboxyethyl phosphine (TCEP; Fluka) in 100mM NH_4HCO_3 for 30 min at 56 °C. Excess TCEP was removed and the gel pieces again dehydrated. Cysteine groups were carbamidomethylated with 100 mM iodoacetamide (Sigma) in 100 mM NH_4HCO_3 for 30 min at room temperature in the dark. After carbamidomethylation the gel pieces were dehydrated and washed with 50 mM NH_4HCO_3 followed by another dehydration step. Proteins were digested by rehydrating the gel pieces in trypsin (Promega) solution (20 mg/L) and incubating at 37 °C overnight. Peptides were

extracted from the gel pieces with 50 µL 0.1% trifluoroacetic acid (TFA) (Sigma). The samples were dried down and 50 µL of water was added and concentrated to less than 20 µL to remove residual NH_4HCO_3. The extracted peptides were concentrated on a C18 ZipTip® and eluted directly onto the MALDI source plate with cyano-4-hydroxycinmamic acid (Fluka), 5 g/L matrix in 66% acetonitrile, 0.1% TFA, 10 mM $NH_4H_2PO_4$ (Fluka).

Mass Spectrometry. Mass spectrometry was performed with a 4800 MALDI TOF/TOF (Applied Biosystems). All MS spectra were recorded in positive reflector mode. Spectra were generated with 500 laser shots/spectrum at laser intensity of 4000 (arbitrary units) with a grid voltage of 16 kV. All peptide-containing spots were internally calibrated using trypsin autolytic fragments.

Database Analysis. Database interrogation was performed with the Mascot algorithm using the MSDB, Swissprot and NCBI databases on a GPS workstation. Search parameters were as follows: Species – Homo sapiens, Enzyme – trypsin; Maximum number of missed cleavages -1; Fixed modifications – carbamidomethyl (C); Variable modifications oxidation (M); N-terminal carbamyl; K-carbamyl; N-terminal and K-carbamyl , precursor tolerance - 100 ppm, fragment tolerance – 0.2 da.

Results

Subjects. Twenty HIV-positive subjects and 31 HIV-negative subjects met the clinical and demographic criteria and agreed to participate in this study. They all signed the consent form and filled in the given health questionnaire. Only 23 of the HIV-positive samples and 13 of the HIV-negative samples met the biochemical inclusion criteria of the study and only these samples were used for further analysis.

2D-Gel Analysis. 2D-gels were prepared as explained. Thirteen gels were run from each group. Only six from the HIV-negative group and eight gels from the HIV-positive group were of sufficient quality for analysis. 7 659 spots were detected in the 14 gels and 562 matches were made. Eleven spots were found to be significantly different in the two groups. One spot (386) was only present on the HIV-positive gels. Three spots (418, 439, 482) were present only on the HIV-negative gels. Seven spots (44, 59, 92, 104, 116, 218, 220) were present on both gels, and all except one (104) were found to be down-regulated in HIV-positive

subjects compared to HIV-negative subjects. Figure 1 shows a representative gel from each group with the relevant spots labelled. Details of each significant spot are given in Table 1.

Protein Identification by MS. The analysis of the samples submitted showed that spectra of suitable quality were obtained for the analysis. A bovine serum albumin control digest indicated that the digestion process was successful with the high concentration but the lower concentration could not be identified with all the databases used. The blank gel control showed only the expected trypsin and matrix crystals. A non-redundant database search was performed which allowed all the samples to be identified with high confidence. Full results are shown in Table 2.

Discussion

The aim of this study was to compare the serum proteome of HIV-positive and HIV-negative subjects. We were able to identify eleven differentially expressed proteins, all fulfilling diverse functions in different tissues. Three of these proteins are well known plasma proteins: α_1-acid glycoprotein (A1AG), haptoglobin and apolipoprotein A-1 (ApoAI). Two proteins form part of the cytoskeleton: type II cytoskeletal keratin 1 (T2CK1) and abnormal spindle-like microcephaly-associated protein (ASMP). One protein is found exclusively in erythrocytes (haemoglobin beta chain), one protein is involved with protein synthesis (60S ribosomal protein L4), and the other two identified proteins are intracellular proteins with, as yet, uncertain roles (coiled-coil domain-containing protein 49 and zinc finger CCCH domain-containing protein 13). Several of these proteins have been reported previously to vary with HIV, while with others, this is the first such report.

A1AG, ApoAI and haptoglobin are acute-phase proteins. An acute-phase protein is defined as a plasma protein whose concentration increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) by at least 50% during an inflammatory reaction.[17] They have been well studied previously as a means to monitor HIV infection. Haptoglobin, CRP and A1AG have been found to increase in HIV-positive patients as the CD4 count falls, while ApoAI levels have been found to decrease.[18-21] Elevated levels of acute-phase proteins can also be found in asymptomatic HIV-positive patients. Samples with an elevated CRP were excluded

from our analysis to help ensure only Stage I disease was studied. Yet, despite a normal CRP, changes in other acute-phase proteins were detected. This discordance between the plasma concentrations of different acute-phase proteins is not uncommon. This is because the components of the acute-phase response are individually regulated.[22]

A1AG and haptoglobin play a greater role than simply markers of inflammation. Haptoglobin is a haemoglobin-binding protein that occurs in three common phenotypes: Hp1-1, Hp2-2 and the heterozygous Hp2-1.[23] Viral loads have been found to be significantly higher in Hp2-2 individuals than in Hp1-1 controls,[24] and CD4 counts have been found to be significantly lower.[25] Haptoglobin has also been reported to be one of the first acute-phase proteins to rise during HIV infection.[20] A1AG, also called orosomucoid, is a small glycoprotein whose physiologic role is still unclear. It is thought to function mainly as a carrier protein for basic and neutral lipophilic endogenous compounds or xenobiotics. Two A1AG proteins are encoded for (ORM1 and ORM2) with slightly different drug-binding properties.[26, 27] A1AG has been found to bind to both the HIV envelope and to macrophage chemokine receptor CCR5, a major co-receptor for HIV, and thus may play a role in the pathogenesis of HIV.[28, 29] A1AG levels are known to be 50-60% higher in HIV-infected people than non-infected controls.[30] It is quite promising that these two proteins, having known HIV associations, were detected as being significantly different. What is unexplained, is that these proteins were found to be decreased in HIV-positive samples compared to controls, as most other studies have found higher levels in HIV-positive people.

Lipid abnormalities are well-known to occur in HIV infection. HIV dyslipidaemia is characterised mainly by decreased plasma concentrations of total, low-density lipoprotein, and high-density lipoprotein cholesterol, and elevated levels of plasma triacylglycerides.[31, 32] In our study, ApoAI was found to be down-regulated in HIV-positive patients. This result is in keeping with the results of a previous study that compared the proteome of patients with AIDS to HIV-negative controls.[33] In that study, it was found that ApoAI was down-regulated in AIDS and this was validated by both Western blot analysis and real time polymerase chain reaction on ApoAI mRNA. In another study, ApoAI was shown to correlate with CD4 count.[34] ApoAI

and HDL cholesterol also return to their pre-disease values with antiviral treatment.[34, 35] Based on our study and this finding, ApoAI is a candidate biomarker for HIV.

ASMP is an intracellular cytoskeletal protein that is predicted to be involved in mitotic spindle function during mitosis.[36] Homozygous mutations of the ASMP gene are the most common cause of autosomal recessive primary microcephaly.[37] Although there has been no reported association between HIV and ASMP, it is interesting to note that Hepatitis C virus has been shown to down-regulate the expression of ASPM,[38] and ASPM is a biomarker for hepatocellular carcinoma.[39] This protein was identified in three different spots in the gel. ASPM is known to occur as various splice variants and isoforms, and to bind a range of cytoskeletal proteins.[36] This may explain its occurrence in multiple positions in the gel, but more detailed structural analysis is required in order to resolve this issue.

The two upregulated proteins were 60S ribosomal protein L4 and coiled-coil domain-containing protein 49. Very little literature is available on these two proteins and this is the first report of a possible relationship with HIV. Another novel potential marker discovered was zinc finger CCCH domain-containing protein 13. Again, very little literature is available on this protein, and there are no previous reports regarding an association with HIV. Further research is required on the role of these three proteins in health and disease.

T2CK1 is a basic protein which is specifically expressed in the spinous and granular layers of the epidermis. T2CK1 forms half of a heterodimer that assembles into intermediate filaments, thereby imparting mechanical strength to the keratinocytes.[40] Mutations in the gene encoding T2CK1 are responsible for a diverse group of disorders of keratinisation.[41] Although some researchers have investigated keratin expression in HIV,[42, 43] there are no reports of its use as a potential biomarker. However, the abundance of keratin in the skin makes it a likely contaminant both through venipuncture and elsewhere.

Haemoglobin beta chain levels were found to be decreased in HIV-positive subjects compared to HIV-negative subjects. Haemoglobin has previously been used as a

biomarker for HIV. Haemoglobin levels have been shown to fall with CD4 count,[44] and a low haemoglobin level is predictive of death in patients starting antiviral treatment. Haemoglobin levels also rise after commencing antiviral treatment.[45] Not surprisingly, all the previous studies have looked at whole blood haemoglobin and not serum haemoglobin. If serum haemoglobin is a reflection of whole blood haemoglobin (when no haemolytic disease is present) then our results could concur with the other studies. Although no haemolysed samples were used in our study, its high concentration in erythrocytes could make it an easy contaminant.

Conclusion

The biomarker development process consists of four stages: discovery, validation, qualification and implementation.[46] In this study, we have used proteomic methods to complete the discovery phase of biomarker development in early HIV. We have successfully identified a number of candidate proteins which undergo consistent changes in HIV disease, some of which have already been studied as potential HIV biomarkers. The fact that four previously known markers were detected gives credence to our method and provides some validation of our study. We have identified five novel proteins, which have not previously been described to have roles as HIV biomarkers. Although rigorous validation is necessary, this information could be used to develop immunoassays to analyse protein changes as an additional method of monitoring changes in HIV infection in the clinical laboratory.

Acknowledgment

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Tables

Table1. List of differentially expressed proteins

Spot number	N (%Vol)	P (%Vol)	Ratio (N/P)	*p* value
44	0.661	0.336	1.968	0.022
59	0.091	0.017	5.336	0.032
92	0.239	0.056	4.235	0.011
104	0.148	0.357	0.416	6.32×10^{-4}
116	0.723	0.206	3.518	0.003
218	0.201	0.044	4.576	0.045
220	1.210	0.382	3.171	0.007
386	-	0.232	0	0.012
418	0.132	-	-	2.34×10^{-5}
439	0.372	-	-	4.17×10^{-4}
482	0.121	-	-	0.007

N – HIV-negative group; P – HIV-positive group

Table 2. Identification of differentially expressed spots

Spot number	Protein	NCBI*	MSDB*	Protein score
44	Abnormal spindle-like microcephaly-associated protein	55663315	AAN40011	134
59	Keratin, type II cytoskeletal 1	7331218	AAF60327	67
92	Abnormal spindle-like microcephaly-associated protein	24061713	AAM44119	146
104	Coiled-coil domain-containing protein 49	8923271	Q9NXE8	125
116	Alpha-1-acid glycoprotein 2	29170378	OMHU2	138
218	Abnormal spindle-like microcephaly-associated protein	55663315	AAN40011	114
220	Haptoglobin	758071	HPHU1	149
386	60S ribosomal protein L4	22002063	T09551	95
418	Zinc finger CCCH domain-containing protein 13	122889118	Q8NDT6	63
439	Apolipoprotein A-I	178775	CAA00975	123
482	Haemoglobin subunit beta	56749856	AAD19696	111

*Accession numbers

Figures

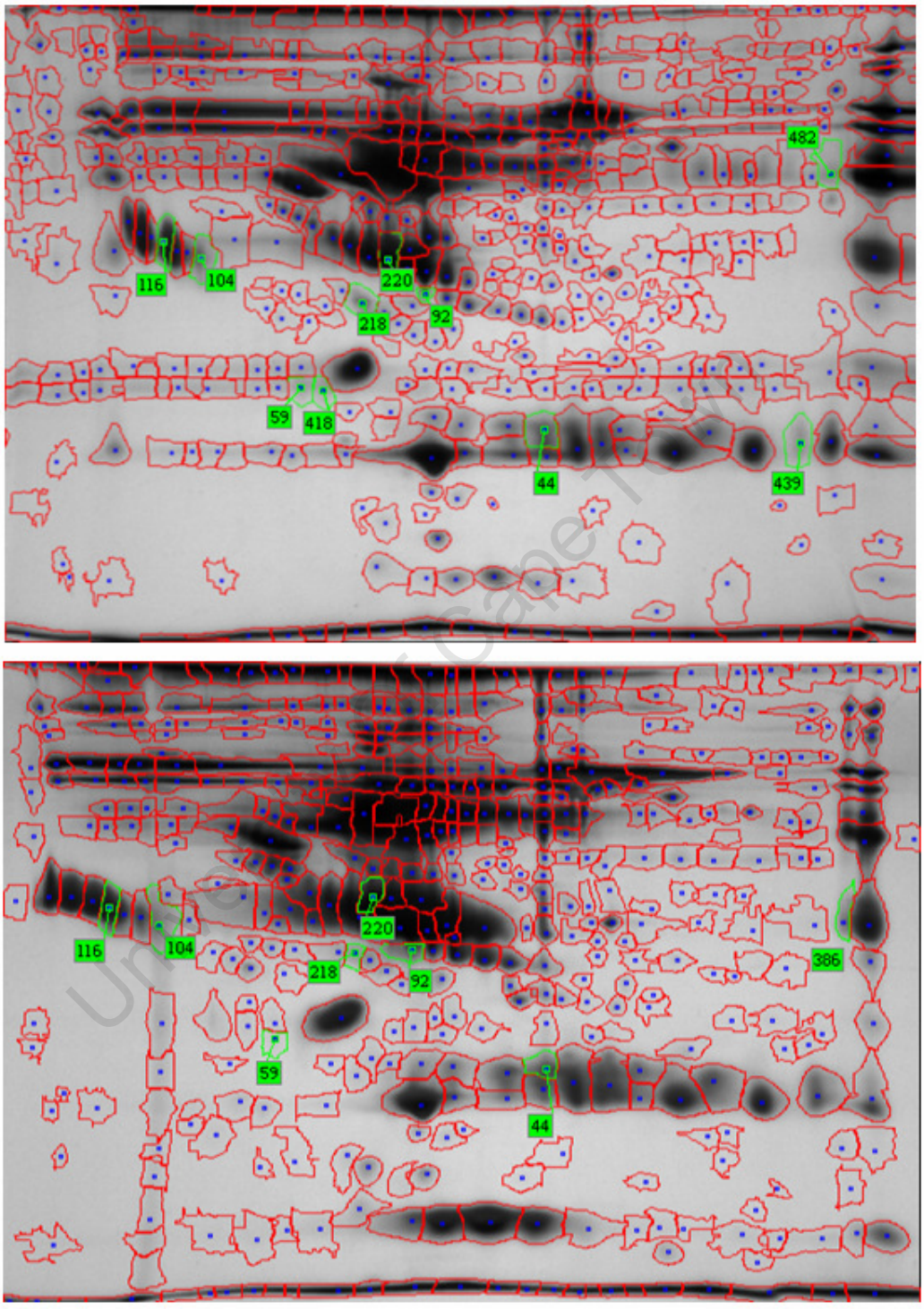

Figure 1. Representative 2D-gels from each group

(Caption on next page)

Figure 1. Representative 2D-gels from each group

Sample gels from HIV-negative (top) and HIV-positive (bottom) groups with significant spots labelled.

Increased in HIV-positive group: 104 - Coiled-coil domain-containing protein 49, 386 - 60S ribosomal protein L4.

Decreased in HIV-positive group: 44, 92, 218 – ASMP, 59 – T2CK1, 116 – A1AG, 220 - Haptoglobin, 439 - ApoAI, 418 - Zinc finger CCCH domain-containing protein 13, 482 - Haemoglobin subunit beta.

Supporting Information

Health Questionnaire

DEPARTMENT OF CHEMICAL PATHOLOGY

UNIVERSITY OF CAPE TOWN

HEALTH QUESTIONNAIRE

BIOMARKER DISCOVERY IN HIV/AIDS USING PROTEOMICS

Visit date: _____

This study is only open to Xhosa males between 21 and 35 years of age.

Questions

1. Are you on any prescription medication?
2. Have you ever been diagnosed with:
    a. Diabetes?
    b. TB?
    c. Asthma?
    d. High blood pressure?
    e. Jaundice?
    f. Epilepsy?
3. Have you had an operation in the last 12 months?
4. Do you suffer from shortness of breath while walking?
5. Do you wake up during the night with difficulty in breathing?
6. In the last three months:
    a. Have you had a cough?
    b. Have you had diarrhoea?
    c. Have you had any pain with urination?
7. Have you ever coughed up blood?
8. Have you been losing weight recently (without trying)?
9. Are you currently suffering from fever, chills or night sweats?
10. Do you have any skin or mouth sores that will not heal?

If you have answered YES to any of the above questions, then you are unfortunately NOT suitable for the study.

Thank you for participating in this study!

Phlebotomy done: _____	_____	_____	
Date	Time	Signature	
Bloods collected: _____	_____	_____	
Date	Time	Signature	

Journal of Proteome Research: Instructions to Authors

The text below includes the table of contents and the section entitled *Preparation of Manuscripts* from the Instructions to Authors document released by the *Journal of Proteomic Research*. The full text can be downloaded at: http://pubs.acs.org/paragonplus/submission/jprobs/jprobs_authguide.pdf

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Instruction to Authors

(Revised January 2010)

Contents

The *Journal of Proteome Research* invites contributions of original research in all aspects of global protein analysis and function. The research should emphasize synergy between physical and life sciences that results in a multidisciplinary approach to the understanding of biological processes. In the selection of manuscripts for publication, the Editors place emphasis on the quality and originality of the work as well as on the breadth of interest to readers and subscribers.

Manuscripts can be submitted as Articles, Letters, Perspectives, Technical Notes, Tutorials, Rapid Communications, and Reviews. Reviews are to be comprehensive, critical accounts of work in a given area.

Authors and reviewers are encouraged to consider the guidelines governing the analysis and documentation of peptide and protein identifications, which were drafted during the Paris workshop on proteomics standards in May 2005. To access these guidelines, go to the *Journal of Proteome Research* homepage. Click Submission & Review, then Information for Authors, then Proteomics Guidelines.

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Elements of a Manuscript

Title and author list

Titles should clearly and concisely reflect the emphasis and content of the paper. Titles are of great importance for current awareness and information retrieval and should be carefully constructed for these purposes. Be consistent in author designation; supply first name, middle initial, and last name for complete identification. At least one author must be designated with an asterisk as the author to whom correspondence should be addressed; telephone and fax numbers and an email address must be listed for the corresponding author. All other authors are encouraged to supply contact information.

All authors' names that are listed must be the same as what is listed on the Paragon Plus submission page; i.e., all authors' names must be entered into the webpage also. With very few exceptions, request for changes or additions of authors will not be approved after acceptance of the manuscript by the Editor.

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All manuscripts must contain an abstract (200 words maximum) that should state briefly the purpose of the research, principal results, and major conclusions.

Keywords

Please include keywords to help categorize the manuscript. Author(s) should supply no more than 10 keywords per manuscript.

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The Journal of Proteome Research features a Table of Contents (TOC) Synopsis that contains a graphic presentation and synopsis for each entry, in addition to the title and author list. Author(s) are required to submit a synopsis (70 words maximum) describing the salient features of the research accompanied by an informative diagram or illustration. The use of colour to enhance the quality and appearance of the graphic is strongly encouraged. **Synopsis artwork should be no wider than 9.0 cm and no taller than 5.0 cm**. The graphic file will be reproduced at 100% of the submission size and should be saved as a TIFF at 300 dpi for colour and at 1200 dpi for black and white. A surrounding margin will be added to this width and height

during production. The TOC graphic and synopsis should be appended as the last page of the word-processing file that is submitted online.

Manuscript contents

All sections of a paper must be presented in a clear and concise manner. Indicate the breakdown among and within sections with centre heads and side heads. Results and Discussion follow the Experimental Section. Keep all information pertinent to a particular section and avoid repetition.

Introduction

The introduction should state the purpose of the investigation and should include appropriate citations of relevant, precedent work but should not include an extensive review of marginally related literature. If the manuscript describes a new or improved method, a clear comparison with existing methods must be provided (including appropriate literature citations).

Experimental section

Use complete sentences (i.e., do not use outline form). Be consistent in voice and tense. *Apparatus:* List only devices of a specialized nature. *Reagents:* List and describe preparation of special reagents only. Do not list reagents normally found in the laboratory or preparations described in standard handbooks and texts. *Procedures:* Because procedures are intended as instructions to permit work to be repeated by others, give adequate details of critical steps. Published procedures should be cited but not described, except where the presentation involves substantial modifications. Very detailed procedures should be presented in a Supporting Information section. *Safety considerations:* **Describe in detail all safety considerations, including procedures that are hazardous or require special precautions and reagents that are toxic, so that laboratory workers who repeat the experiments can take appropriate safety measures. Include procedures and references for the neutralization, deactivation, and ultimate disposal of unusual byproducts.** *Results and Discussion:* The results may be presented in tables or figures; however, many simple findings can be described directly in the text with no need for tables or figures. The discussion should be concise and present an interpretation of the results. In most cases, combining results and discussion in a

single section will give a clearer, more compact presentation. *Test subjects:* It is the responsibility of the author(s) to obtain appropriate institutional approval for the use and generation of data from animal and human samples.

Conclusions

Use the conclusion section only for interpretation and not to summarize information already presented in the text or abstract.

Tables

Tables should be numbered consecutively with Arabic numerals and should be grouped at the end of the paper. Each table should be provided with a descriptive heading, which, together with the individual column headings, should make the table as self-explanatory as possible. Footnotes in tables should be cited in the table by italic superscript letters. The sequence of letters should proceed by line rather than by column. If a reference is cited both in the text and in a table, insert a lettered footnote in the table to refer to the numbered footnote in the text. When formatting a table, author(s) are requested to keep in mind the type area of the journal page (17.8 × 25.0 cm) and the column width (8.5 cm) and to make tables conform to the limitations of these dimensions. When data are arranged into columns, space should be used efficiently.

References

Number literature citations and explanatory notes in one consecutive series by order of mention in the text; use superscript Arabic numbers without parentheses. All references should be grouped at the end of the manuscript. Author(s) should consult *The ACS Style Guide, 3rd ed.* (ACS: Washington, DC, 2006) for the appropriate style to use in citations of journal papers, books, and other publications. In literature references, journal abbreviations should be those used by Chemical Abstracts. In the web edition of the paper, references will be linked to various electronic sources (the corresponding abstract from Chemical Abstracts Service, full text from other ACS journals, etc.); therefore, the accuracy of the references is critical. Author(s) are responsible for the accuracy of the references.

Nomenclature

Registered trademark names should be capitalized whenever used. Usually the chemical name or composition should be given in parentheses or in a reference at the first occurrence of such a name. Chemical Abstracts (CA) nomenclature rules are described in Appendix IV of the Chemical Abstracts Index Guide. For CA nomenclature advice, consult the Manager of Nomenclature Services, Chemical Abstracts Service, P.O. Box 3012, Columbus, OH 43210-0012. A name-generation service is available for a fee through CAS Client Services, 2540 Olentangy River Rd., P.O. Box 3343, Columbus, OH 43210-0334 (answers@cas.org). Avoid trivial names. Well-known symbols and formulas may be used if no ambiguity is likely. Define trade names, abbreviations, and acronyms at point of first use.

Artwork

Artwork should be sequentially numbered using Arabic numbers. Schemes and charts may have titles and footnotes; figures should have captions. Structures should be designated with boldface Arabic numbers. Author(s) should remove all colour from graphics, except graphics that they would like to be considered for publication in colour (see Colour section for details). When inserting illustrations in the manuscript, author(s) should follow the web instructions for manuscript preparation. For additional guidelines, see "Working with Graphics", which can be accessed by going to http://pubs.acs.org/jpr and clicking on Submission & Review/Info for Authors & Reviewers/Information for Authors/Manuscript Preparation and Submission. Please note that the quality of the final illustrations depends on the quality of the original artwork provided. Figures cannot be modified or enhanced by the journal production staff.

Artwork should be provided at the size it will be printed in the journal. To ensure good quality production in the journal, the author(s) should print a copy of each graphic (at the size desired in the final printed version) to verify that all parts of the artwork are clear and legible. Any changes to the artwork (including size changes) must be made in the drawing program that was used to create the artwork, and the revised artwork must be reimported into the manuscript. Artwork should be constructed in keeping with the journal column widths—one column (which is preferred): maximum of 8.5 cm (3.3 in.); two columns: minimum of 10.5 cm (4.1 in.)

and maximum of 17.8 cm (7.0 in.); total height for one or two columns: 24 cm (9.5 in.).

In preparing artwork, ensure that

- the lettering is 4.5 pt or larger and is in Helvetica or Arial font,
- the lines are no thinner than 0.5 pt,
- the lettering and the lines are of uniform density, and
- dark black ink is used to achieve the best contrast.

Use a simple cross-hatch design to show a pattern or to achieve a 3-D effect. Do not submit colour prints when the artwork will be reproduced in black and white.

Supporting Information

Almost any type of supplementary figures or data (reproductions of spectra, experimental procedures, tabulated data, expanded discussion of peripheral findings, etc.) can be accommodated in Supporting Information. The author(s) should include a Supporting Information Available statement at the end of the manuscript. Use the following format: "Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org."

Supporting Information must be submitted at the same time as the manuscript and uploaded separately to the ACS Paragon Plus Environment. A list of acceptable file types is available on the Web. All Supporting Information files of the same type should be prepared as a single file (rather than submitting a series of files containing individual images or structures). For example, all Supporting Information available as PDF files should be contained in one PDF file.

Do not upload figures and tables that are to be published in the article into the Supporting Information file.

Manuscript types

The research pages of the *Journal of Proteome Research* are devoted to scholarly publication encompassing all aspects of systems-oriented protein analysis and function. Papers that integrate fields such as chemistry, mathematics, applied

physics, biology, and medicine in order to provide better understanding of the function of proteins in biological systems are encouraged. Articles may focus on any phase of proteome research, including sample preparation, separation, characterization, and analysis of proteins (including informatics), as well as the development of new methodologies. Papers involving extensive use of computers and data processing (e.g., bioinformatics) will be judged by the usual criteria of originality, technical content, and value to the field. Detailed mathematical derivations, computation procedures, and programs should be presented as Supporting Information. If the research involves the use of human or animal samples, the author(s) must have permission from their institutions and must conform to commonly practiced ethical standards.

In addition to regular research papers, Letters, Technical Notes, Perspectives, Reviews, Tutorials, and Rapid Communications are published.

Letters are opinion pieces related to the field of proteomics. Letters can be supported by brief descriptions of scientific data and references, but such information is not necessary for acceptance.

Technical Notes should be brief descriptions of novel apparatus or techniques that offer definite advantages over those already available. These papers should demonstrate real ingenuity on the author(s)' part. Technical Notes have a maximum length of 8 journal pages (8000 words).

Perspectives articles are unlike others in the research section in that they are not reports of original research or reviews with the traditional objective of summarizing progress in a field. Instead, Perspectives point out the author(s)' vision of the character and importance of a new direction in proteome research. They are aimed at specialists and experts in the field, and the level of writing reflects their sophistication with the topic. They are not intended to be accounts or analyses of an individual's personal research. Perspectives can be submitted without invitation; some will be invited. Author(s) are encouraged to suggest experts in the field who can review their Perspectives. Because only a limited number of Perspectives will be published, the Editor recommends a preliminary contact by prospective author(s).

Editorial decisions will be based on technical quality, significance, and demonstration of a new direction in proteome research.

Reviews are thoroughly documented, peer-reviewed assessments of selected areas of proteome research literature for the purpose of identifying critical research needs. Reviews can be longer than the typical research article but should generally be limited to 9000 words, including references, graphs, and figures. Critical reviews should increase readers' knowledge through discriminating comparisons and insightful organization of the material. A mere listing of literature citations with descriptive comments is inadequate. Criteria for acceptability include current importance of the field under review, thoroughness of the literature coverage, clarity of text, and clear identification of research needs. Tutorials are articles written for researchers who are new to the field of proteomics. These articles cover timely topics but do not necessarily present new data. Compared to Reviews, Tutorials are narrower in scope and should more fully explain the relevant background of the topic. The editors consider these articles important for the journal's educational mission.

Tutorials are typically 3000—6000 words long, though they could be longer in some cases. Although many Tutorials are invited, unsolicited submissions will be considered.

Rapid Communications are concise accounts of work that is especially time-sensitive. To expedite the handling of these manuscripts, authors should take care to submit papers that will not require revision. In addition, ~2 weeks before submitting a paper, the corresponding author should contact the editorial office with the names of suggested reviewers.

File preparation for manuscript revisions

Author(s) are encouraged to use the template for their electronic version. In manuscript preparation, use the document mode or its equivalent in the word-processing program; that is, do not save files in "Text Only" (ASCII) mode. If a non-Western version of a word-processing program was used to prepare the manuscript, save the file in rich-text format (RTF). The text should be left-justified, and

automatic end-of-line hyphenation should be turned off. Use “returns” only to end headings and paragraphs, not to break lines of text. Do not insert spaces before punctuation. References must conform to the format printed in the journal. Characters must be correctly represented throughout the manuscript: for example, 1 (one) and l (ell), 0 (zero) and O (oh), x (ex) and × (multiplication sign). Check the final copy carefully for consistent notation and correct spelling. The Editorial Office conversion program will faithfully translate any errors present in a file. The complete manuscript should be placed in one file created in one of the word-processing programs listed below. All graphics in this file must be given at the size they are to be printed in this journal. Proof a printout of the manuscript from a 600-dpi laser printer to ensure that all artwork is clear and legible.

Acceptable word-processing packages

For a list of currently acceptable software and file designations, please refer to the submission guidelines presented at http://paragonplus.acs.org.

PART D

University of Cape Town Dissertation Guidelines

Minimum Requirements for Dissertations for MMed and MPhil for Subspecialities Degrees

Following extensive discussion with Heads of Divisions, Dr S Kalula and Prof S Kidson recommend the following minimum criteria for dissertations for MMed and MPhil (subspeciality) degrees:

The MMed minor dissertation (or the MPhil dissertation in the case of sub-specialities) is one of three examination components of the MMed/MPhil degree. This minor dissertation carries one third of the weight of a full master's dissertation in terms of its credit weighting.

The dissertation must be a study containing the results of an analytical, quantitative, or
epidemiological study carried out by the candidate (for certain disciplines, the candidate may chose instead to do a qualitative study, an audit cycle or a formal systemic review). A case report is not acceptable for the dissertation.

The dissertation must be the result of independent work of the candidate conducted under the guidance and direction of a supervisor(s) and should demonstrate evidence of an ability to undertake research, to adequately interpret results and to comprehensively and critically review the relevant literature. Although the findings of the research need not necessarily be original, they must be seen to advance scientific understanding. The topic and scope of research will depend on the particular disciplines and must be agreed upon in consultation with the supervisor(s).

Research Protocol

Candidates intending to register for the MMed/MPhil Part III are required to submit a full research protocol for approval to their respective Departmental Research

Committee (DRC). The candidate must also obtain FHS UCT Ethics approval prior to conducting their research. This full research protocol (together with a copy of the ethics approval letter) must be submitted to the postgraduate administration for approval by the Board of the Faculty of Health Sciences, prior to commencement of the research. For most disciplines, submission of the research protocol should be made no later than the end of year 2.

The research protocol should outline the scope and content of the dissertation and must include the title of the proposed dissertation, name of the supervisor(s) and their brief curriculum vitae.

Submission of Dissertations

On completion, the dissertation should be submitted to the Faculty Postgraduate Officer. The candidate should inform the Faculty Officer one month in advance of the intention to submit.

Submission deadlines:

1. March 15th for June graduation
2. August 15th for December graduation

Supervisors will be requested by the Faculty Postgraduate Officer to submit a letter supporting submission. This letter should be supplied by the primary supervisor. If this supervisor is external, the internal supervisor must be kept informed at every stage of the process. Specific submission requirements may be set by individual disciplines.

Note on fees: To avoid attracting fees, dissertations need to be submitted before the beginning of the first quarter (first day of academic year), and before the start of the second semester (mid July) to qualify for a 50% fee rebate.

SUPERVISORS

One cannot overemphasize the importance of identifying a dissertation supervisor as early as possible. The supervisor should be an individual who can relate to the candidate's research project, be available for frequent and regular discussion and

advice, and someone with whom the candidate can develop a good working relationship. Where specialised equipment and/or laboratory work is required for the study, the supervisor should assist in facilitating such access to such facilities. Supervisors may assist candidates in developing scientific communication skills but they are not required to do detailed editing or correction of spelling, grammar, or style. They may refer candidates to the UCT Writing Centre for this purpose.

The primary supervisor may be based outside the candidate's home department, faculty or university. In such a case, an internal (or secondary) supervisor will be required in addition to the primary supervisor, to serve as a guide and link to discipline-specific procedures. Primary supervisors retain responsibilities to the candidate and the university until the dissertation process is complete.

Please note: in order to assist a candidate with a master's research topic the supervisor needs to hold a master's degree or higher, or have relevant research experience. If the primary supervisor does not hold a higher degree or equivalent (such as a Fellowship of The College of Medicine of South Africa), then a secondary supervisor who has a higher degree will need to be appointed in addition to the primary supervisor.

Candidates are strongly encouraged to publish the study with the supervisor(s) as co-author(s). This may require work beyond the graduation date. Such arrangements should be discussed and documented in advance.

The Dissertation

Submission of the dissertation should satisfy the following criteria:

1. The title page should contain the candidate's name, dissertation title and the name of the university. It must also state the degree, e.g. Master of Medicine (MMed) in Public Health Medicine, Occupational Medicine, Family Medicine, Surgery, etc. The title page should also include a statement to the effect that the research report is based on independent work performed by the candidate and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university. It must

also state that this work has not been published *prior to registration* for the abovementioned degree.

2. The body of the dissertation, which must be structured in 4 parts, should include the following:

   Part A: The *protocol* (as approved by the Departmental Research Committee and Faculty Research Ethics Committee). The protocol should not exceed 4000 words.

   Part B: A *structured literature review* appropriate to the subject matter and methods of the dissertation. The literature review must, amongst other things, show that the student is sufficiently acquainted with the relevant literature and is able to perform a critical appraisal and, if appropriate for the topic, show a good understanding of evidence-based medicine.

   The review should be between 3 000 and 4 000 words.

   A suggested structure for the literature review is as follows:

   a) Objectives of literature review
   b) Literature search strategy, including inclusion and exclusion criteria
   c) Quality criteria - some leeway will be allowed here, as candidates will vary in their ability to appraise studies. This will also vary with the nature of the dissertation.
   d) Summary or interpretation of literature
   e) Identification of gaps or needs for further research
   f) References (which will overlap with but will not be the same lists as in the journal article and protocol)

   Part C: The results of the study must be presented in the form of a *manuscript* of an article for a named peer reviewed journal, meeting all the requirements set out in the "Instructions for Authors" of that journal, including the word count and referencing style. (Unless specially motivated, the journal chosen will need to allow for *at least* 3000 words excluding abstract, tables, figures

and references). The "Instructions to Authors" of the journal must be appended. The journal chosen for publication must be appropriate to the subject matter of the dissertation and accredited by the Department of Education or listed in the citation index of the Institute for Scientific Information (ISI).

Important note: the candidate need not have submitted the article, not is the acceptance of the article and requirement for passing the degree. The norm of practise is to publish the study with the supervisor(s) as co-author(s) and candidates are strongly encouraged to submit their manuscript either before or after examination of the mini-dissertation.

Part D: All supporting documents including:

- Questionnaire/data capture instrument
- Consent forms and any related participant information sheets
- Technical appendices, including, if considered necessary, any additional tables not included in the main manuscript for the examiner to have available. These should be accompanied by a brief narrative.
- Official Ethics approval letter from the Faculty Research Ethics Committee

3. The article does *not* have to be submitted to the journal in order to meet academic requirements.

4. A candidate must submit 2 copies of the dissertation in temporary binding, and an electronic copy on compact disc in a universally readable format (e.g. pdf).

Examiners

The full dissertation will be submitted for examination through the Postgraduate office of our Faculty to two external examiners (nominated by the supervisors and HOD).Three examiners will be nominated, two of which are invited to examine, and one held as an alternate. All examiners must be external to UCT. These nominations are circulated to the Faculty Dissertation Committee. It is the *supervisor's (or co-*

supervisor's) responsibility to submit names of potential examiners to the Faculty Officer when the candidate is ready to submit.

The examiners will be well briefed regarding the specific requirements and criteria for submission and examination of the mini-dissertation. Such criteria will clearly explain the difference between the minidissertation and a Master's degree by dissertation alone.

Details required for each examiner are: academic qualifications, postal and/or physical address, telephone and fax numbers and e-mail address, and one paragraph description of their standing in the relevant field (drawn from their CV if need be.)

The candidate may not be informed of the identity of the examiners. After the outcome of the minidissertation has been finalised, the examiners' identities are made known if the examiners have indicated that they do not object to this.

Consent Form (English)

DEPARTMENT OF CHEMICAL PATHOLOGY

UNIVERSITY OF CAPE TOWN

CONSENT FORM

BIOMARKER DISCOVERY IN HIV/AIDS USING PROTEOMICS

Often, when blood is analyzed from people with a particular disease, differences are noted in various protein concentrations. These concentration differences can sometimes be used to diagnose a disease or tell how serious that disease is. The purpose of this study is to establish whether there are any differences between protein concentrations found in the blood of HIV positive and HIV negative people. These differences may prove to be useful in future HIV testing.

The blood that is being taken from you will be tested for HIV and then analyzed for different protein concentrations.

I, ……, agree to participate in the above study and hereby give permission that blood may be taken and serum analysed and/or stored.

I understand that I will undergo the following investigations:

i. Venous blood will be taken for a HIV test and the measurement of serum proteins
ii. I further understand that a portion of these blood samples will be stored for:
    a) possible reanalysis
    b) analysis when the study is completed, if additional information has become available
    c) further research purposes, subject to the approval of the University of Cape Town Research Ethics Committee and that any information from such research will remain confidential
    d) I understand the results of these tests will not become available to me

I have been informed that:

a) the investigators are under the obligation to respect medical confidentiality
b) No DNA will be stored and no genetic/DNA tests will be done on these samples
c) All samples will be anonymised prior to being sent to the laboratories.

I UNDERSTAND THAT THIS STUDY WILL BE OF NO BENEFIT TO ME AND ONCE MY BLOOD HAS BEEN TAKEN, THERE WILL BE NO WAY TO TRACE IT BACK TO ME.

ALL OF THE ABOVE HAVE BEEN EXPLAINED TO ME IN A LANGUAGE THAT I UNDERSTAND AND MY QUESTIONS HAVE BEEN ADEQUATELY ANSWERED

Participant: Name …… Date: ……..

Participant: Sign ……..…… Date: ……..

Informed consent obtained by: Name …….

Sign …….

Witness: …… Sign: ……..…….

Consent Form (Xhosa)

ISEBE LENZULULWAZI NGEZIFO EGAZINI

IDYUNIVESITHI YASEKAPA

IFORM YOKUCELA IMVUME

UKUPHANDWA NZULU NGESIFO IHIV/AIDS KUSETYENZISWA IPROTEOMICS

Ngamaxesha amaninzi xa kuphononongwa igazi labantu abanesifo esithile, umahluko uyabonakala kwi proteins egazini. Lo mahluko kwezi proteins uyasetyenziswa ukuxela ukuba sikhona esisifo okanye hayi, kwaye ziyakwazi nokubonisa ukuba esisifo singanobungozi kangakanani na. Oluphando nzulu lwenziwayo linganceda ekuhlolweni kwegazi xa kukhangelwa intsholongwane iHIV.

Sicela uthathe inxaxheba koluphando nzulu ngokusivumela sitsale igazi lakho, silihlolele intsholongwane ye HIV siphinde silihlolele ezinye iproteins ezinobakhona egazini, kusetyenziswa iproteomics ngaba baphandi nzulu bangentla.

Mna,……,ndiyavuma ukuthatha inxaxheba koluphando nzulu, ndinikeza ababaphandi imvume youkutsala igazi lam ukuze lihlolwe, liphononongwe kusetyenziswa iproteomics.

Ndixelelwe kwaye ndiyazi ukuba ndizakuhlolwa ngoluhlobo:

iii. Igazi lam lizakutsalwa lihlolelwe intsholingwane iHIV kunye nezinye iproteins ezinokuthi zibekhona egazini.
iv. Kwaye ndiyaqonda ukuba eligazi lizakugcinwa ngaba baphandi nzulu ukwenzela:

a) Liphinde lihlolwe xa ikhona imfuneko yokwenza oku.
b) Liphinde lihlolwe xa oluphando nzulu sele lugqityiwe ukuba zikhona indlela ezintsha ezivelayo zokuhlola igazi.

c) Abanye abaphandi nzulu bakwazi ukulisebenzisa, kodwa phambi kokuba lisetyenziswe, iUniversity of Cape Town Research Ethics Committee kufuneka ibanike imvume nabo benze isithembiso sokuba iziphumo zalamagazi zihlala zilihlebo labaphandi kuphela.
d) Ndixelelwe kwaye ndiyazi ukuba iziphumo nengxelo zalamagazi andizikuzixelelwa.

Ndixelelwe ukeba:

a) Ababaphandi nzulu basenzile isithembiso sokugcina ingxelo, neziphumo zamagazi zilihlebo.
b) Akukho DNA izakugcinwa kwaye akukho phando lweDNA okanye genetics luzakwenziwa kweli gazi.
c) Eligazi lizakugcinwa luyimfihlo yabaphandi nzulu.

NDIYAZI UKUBA OLUPHANDO NZULU ALUZUKUTSHINTSHA NTO KUHLOBO ENDIKHATHALELWA NGAYO KULE KLINIKI KWAYE XA SELE LITHATHIWE AKUSOZE KUBEKHO NDLELA YOUKUTHI LELAM.

ZONKE EZINKCUKACHA ZINGENTLA ZICACISIWE KUM NGOLWIMI ENDILAZIYO KWAYE YONKE IMIBUZO EBENDINAYO IPHENDULWE NGENDLELA EYONELISEKAYO.

Umthati nxaxheba: Igama…… Umhla:……..

Umthathi nxaxheba: Sayina…… Umhla:……..

Imvume youkuthatha inxaxheba icelwe ngu: Igama ……

Sayina …….

Ingqina: ……. Sayina…..…….

Phlebotomy Protocol

DEPARTMENT OF CHEMICAL PATHOLOGY

UNIVERSITY OF CAPE TOWN

PROTOCOL

BIOMARKER DISCOVERY IN HIV/AIDS USING PROTEOMICS

Subjects

- Blood is required from 30 HIV positive and 30 HIV negative individuals.
- Blood is to be collected from Xhosa males aged 21 to 35 years.
- Participants must not be on any medical treatment.
- Participants must not have any significant medical history, in particular participants must be free from:
    - Diabetes
    - Kidney disease
    - Liver disease
    - Heart disease
    - Infection or inflammatory conditions (other than HIV)
    - Any major systemic illness
    - Any signs or symptoms that may indicate an undiagnosed disease.

Phlebotomy

- Blood should ideally be collected from the left cubital fossa in a seated position.
- After the area has been cleaned, a tourniquet may be applied to make venipuncture easier.
- The time between applying the tourniquet and collecting the blood must be minimized.
- Blood must be collected into a 5ml SST (yellow top) tube. The tube must be filled if possible.

Storage and Transport

- Once collected, the blood should be stored at room temperature.
- The blood must reach the laboratory within six hours.

- At the laboratory the serum must be separated (4000rcf for 5 minutes) and frozen at -20ºC.

Thank you for participating in this study!

Please contact me if you have any queries: David Haarburger (021) 404-4135

Ethics Approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: lamees.emjedi@uct.ac.za

17 May 2007

REC REF: 211/2007

Prof TS Pillay
Chemical Pathology

Dear Prof Pillay

PROJECT TITLE: BIOMARKER DISCOVERY IN HIV/AIDS USING PROTEOMICS

Thank you for submitting your study to the Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

Wesley Henley

A/PROF. M. BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

pp

lemjedi

Supplementary Figures

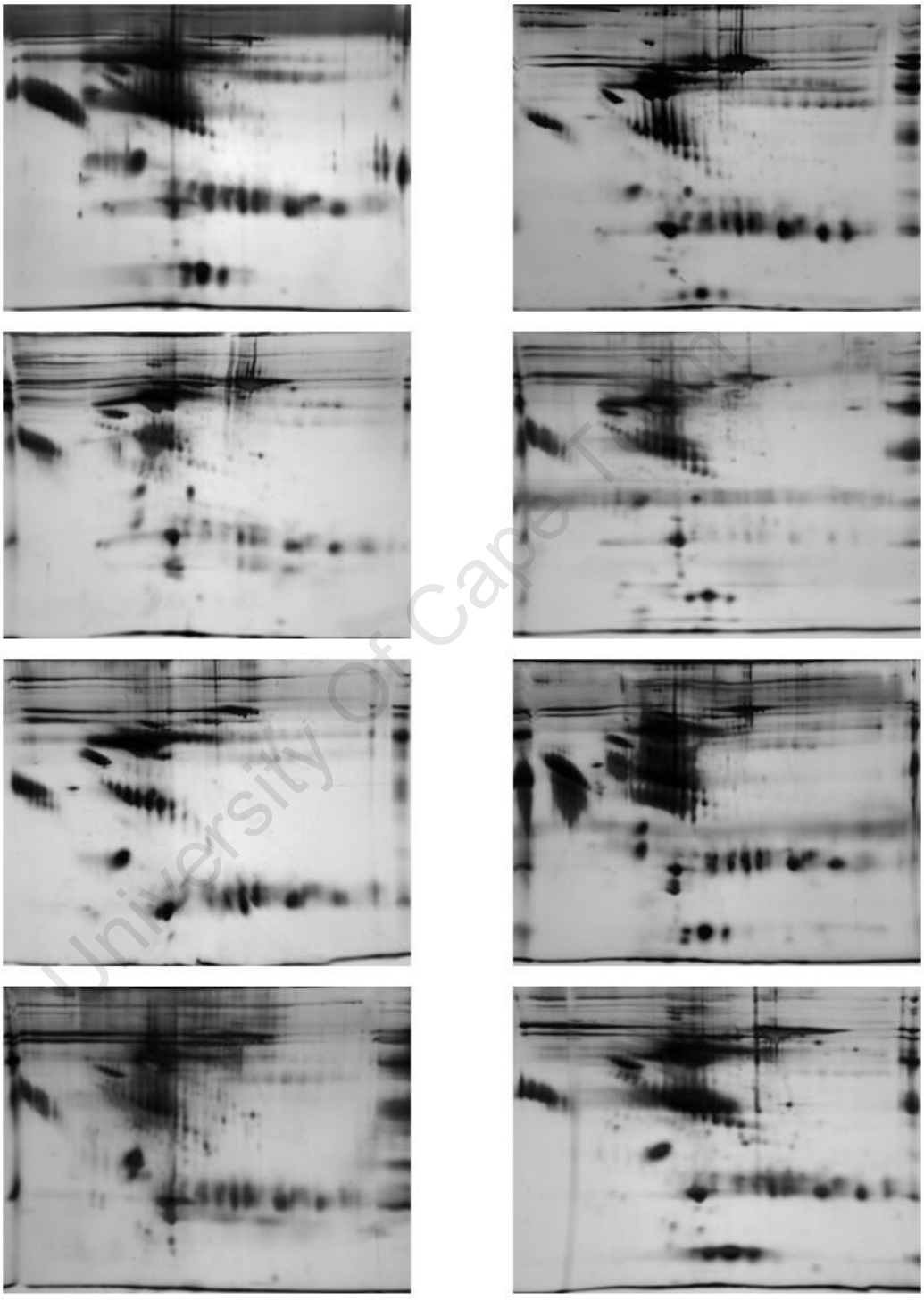

Figure S1. Gels from HIV-positive subjects

Silver-stained two-dimensional electrophoresis gels of HIV-positive subjects.

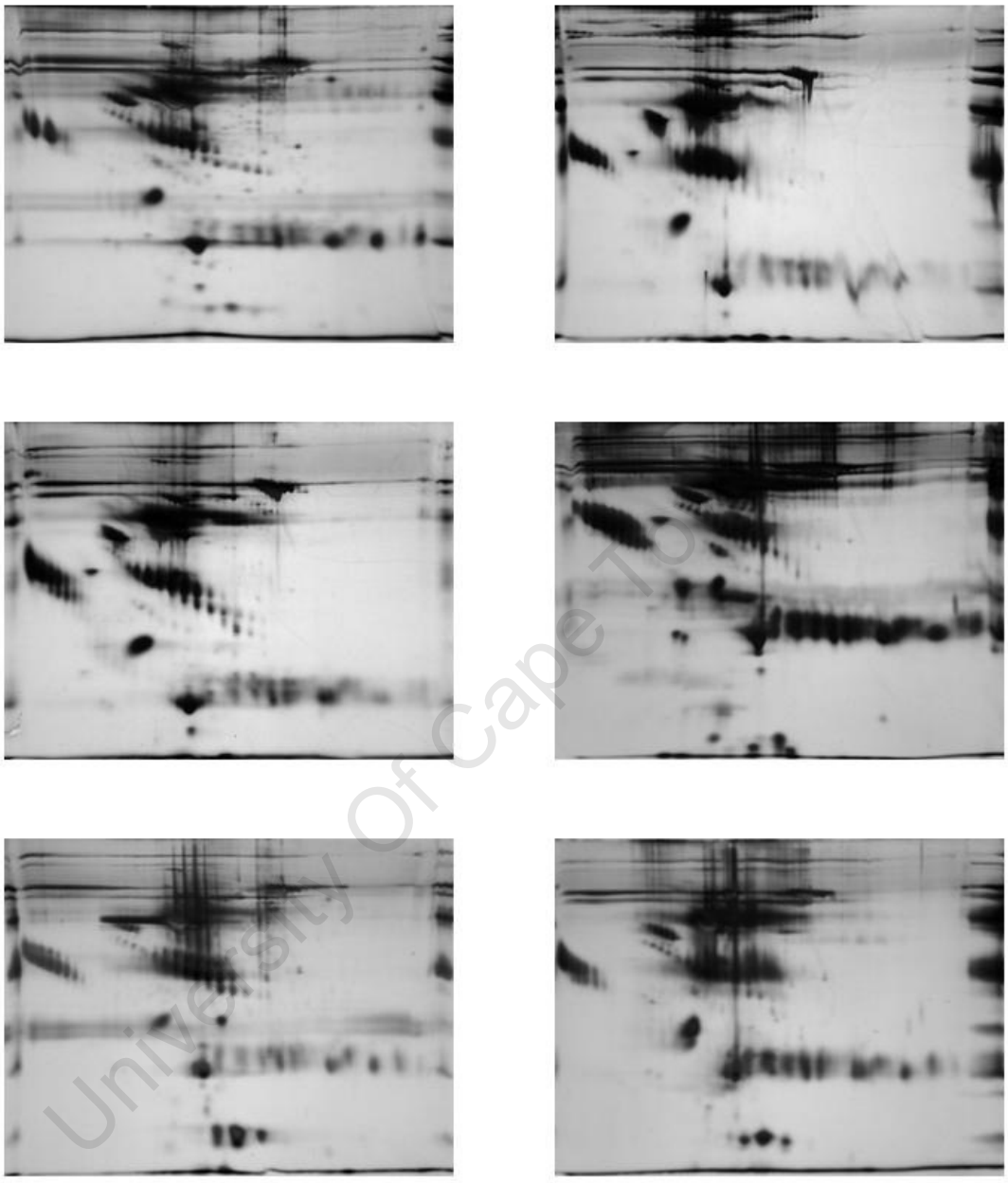

Figure S2. Gels from HIV-negative subjects

Silver-stained two-dimensional electrophoresis gels of HIV-negative subjects.

Mass Spectrometry Data

CPGR | CENTRE FOR PROTEOMIC & GENOMIC RESEARCH

Analysis Information

Report Type	Protein-Peptide Summary by Spot	Analysis Type	Combined (MS+MS/MS)
Sample Set Name	New Sample Set 1	Database	MSDB
Analysis Name	MSDB Homo Sapiens N-terminal carbamyl	Creation Date	10/22/2009 15:40:21
Reported By	10/29/2009 15:38:29 - admin	Last Modified	10/22/2009 16:22:52
MS Acq. : Proc. Methods	(Unspecified) : (Unspecified)		
Interpretation Method	(Unspecified)		

Gel Idx/Pos	32/B5	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	2

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	AF509326 NID: -	Homo sapiens	AAN40011	49	134	100		Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3627	572.3632	0.0005	1	1893	1896	QIRR					Mascot
574.3307	574.344	0.0133	23	1293	1297	EKAAR					Mascot
576.3099	576.3455	0.0356	62	2730	2733	TERK			Carbamyl (N-term)[0]		Mascot
578.333	578.3429	0.0099	17	1992	1995	IMKK			Carbamyl (N-term)[0], Oxidation (M)[2]		Mascot
579.35	579.3345	-0.0155	-27	3417	3420	ILYK			Carbamyl (N-term)[0]		Mascot
580.3201	580.3403	0.0202	35	2946	2949	YRAK			Carbamyl (N-term)[0]		Mascot
581.3041	581.3397	0.0356	61	755	759	AYTAR					Mascot

583.335	583.3223	-0.0127	-22	801	804	HLWK		Mascot
584.3151	584.3516	0.0365	62	2552	2555	EKHK	Carbamyl (N-term)[0]	Mascot
586.3671	586.3554	-0.0117	-20	1329	1333	VLAQR		Mascot
588.3575	588.36	0.0025	4	2184	2187	RTLR	Carbamyl (N-term)[0]	Mascot
589.3304	589.3545	0.0241	41	1680	1684	NATIK	Carbamyl (N-term)[0]	Mascot
590.3442	590.3466	0.0024	4	2751	2755	GMKVR		Mascot
592.3453	592.3432	-0.0021	-4	2922	2926	GFIQK		Mascot
593.3405	593.3502	0.0097	16	2724	2727	LYVR	Carbamyl (N-term)[0]	Mascot
594.3609	594.3496	-0.0113	-19	1280	1283	YKLK	Carbamyl (N-term)[0]	Mascot
596.3627	596.3503	-0.0124	-21	2402	2405	RHLK	Carbamyl (N-term)[0]	Mascot
600.3939	600.3752	-0.0187	-31	2106	2110	GIRVR		Mascot
602.366	602.3752	0.0092	15	1047	1050	LLWK	Carbamyl (N-term)[0]	Mascot
606.3721	606.3562	-0.0159	-26	2678	2682	GFKVR		Mascot
610.3558	610.3564	0.0006	1	2056	2060	AYKTK		Mascot
614.362	614.3647	0.0027	4	740	744	ISVPR	Carbamyl (N-term)[0]	Mascot
615.3685	615.3554	-0.0131	-21	1893	1896	QIRR	Carbamyl (N-term)[0]	Mascot
622.3671	622.3768	0.0097	16	3249	3252	LYKR	Carbamyl (N-term)[0]	Mascot
630.3933	630.3927	-0.0006	-1	2420	2424	SLLVR	Carbamyl (N-term)[0]	Mascot
632.3726	632.3942	0.0216	34	1284	1288	TDLKR		Mascot
633.3929	633.3787	-0.0142	-22	611	616	TSAVKK		Mascot
636.4078	636.3929	-0.0149	-23	885	889	FTLKK		Mascot
644.3838	644.3928	0.009	14	185	189	VDRVR		Mascot
646.4069	646.3989	-0.008	-12	2470	2474	RLMVK		Mascot
647.3511	647.3797	0.0286	44	3164	3168	YHSIK		Mascot
648.4442	648.4175	-0.0267	-41	1367	1371	FLKLK		Mascot
650.3984	650.4178	0.0194	30	2354	2358	YRALK		Mascot
658.4246	658.3967	-0.0279	-42	1716	1721	KIAAQK		Mascot
660.4113	660.3987	-0.0126	-19	1335	1339	LLMLK	Carbamyl (N-term)[0]	Mascot
662.4017	662.4146	0.0129	19	2470	2474	RLMVK	Oxidation (M)[3]	Mascot

	664.3664	664.4156	0.0492	74	2929	2933	FQEIK					Mascot
	666.3569	666.4076	0.0507	76	2006	2011	AYSIGR					Mascot
	678.4045	678.4155	0.011	16	3062	3066	YIRAR					Mascot
	680.3627	680.4255	0.0628	92	1325	1328	YWRR					Mascot
	694.363	694.4268	0.0638	92	2587	2591	YRANK		Carbamyl (N-term)[0]			Mascot
	704.3474	704.4157	0.0683	97	1933	1938	AWTAGR		Carbamyl (N-term)[0]			Mascot
	758.4671	758.4481	-0.019	-25	3092	3097	GWLVRK					Mascot
	785.4417	785.4525	0.0108	14	1216	1221	NFHLVR					Mascot
	786.4396	786.4404	0.0008	1	3399	3404	IYSLYK					Mascot
	807.4327	807.4324	-0.0003	0	2275	2280	TLMMRR					Mascot
	856.5151	856.5213	0.0062	7	3194	3199	HFLLRK		Carbamyl (N-term)[0]			Mascot
	868.4788	868.5237	0.0449	52	1455	1460	EWHLRK					Mascot
	870.4719	870.5401	0.0682	78	3076	3081	YIEFKK		Carbamyl (N-term)[0]			Mascot
	870.4719	870.5401	0.0682	78	3076	3081	YIEFKK		Carbamyl (N-term)[0]			Mascot
	1057.5425	1057.5635	0.021	20	2596	2603	VFQHNELK		Carbamyl (N-term)[0]			Mascot
	1179.5867	1179.5985	0.0118	10	1769	1776	QYYLKMYK		Carbamyl (N-term)[0]			Mascot
2	Similar to asp gene product.- Homo sapiens (Human).							Q8N4D1	29	110	100	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3627	572.3632	0.0005	1	560	563	QRLR					Mascot
574.3307	574.344	0.0133	23	543	547	EKAAR					Mascot
578.3296	578.3429	0.0133	23	825	828	FIQK			Carbamyl (N-term)[0]		Mascot
579.35	579.3345	-0.0155	-27	1082	1085	ILYK			Carbamyl (N-term)[0]		Mascot
580.3201	580.3403	0.0202	35	611	614	YRAK			Carbamyl (N-term)[0]		Mascot
581.3041	581.3397	0.0356	61	5	9	AYTAR					Mascot
583.335	583.3223	-0.0127	-22	51	54	HLWK					Mascot

586.3671	586.3554	-0.0117	-20	579	583	VLAQR					Mascot
588.3463	588.36	0.0137	23	515	518	KEIR	Carbamyl (N-term)[0]				Mascot
590.2892	590.3466	0.0574	97	1051	1055	ASDVR	Carbamyl (N-term)[0]				Mascot
594.3609	594.3496	-0.0113	-19	530	533	YKLK	Carbamyl (N-term)[0]				Mascot
600.4191	600.3752	-0.0439	-73	894	898	IKAIR					Mascot
602.366	602.3752	0.0092	15	297	300	LLWK	Carbamyl (N-term)[0]				Mascot
615.3685	615.3554	-0.0131	-21	560	563	QRLR	Carbamyl (N-term)[0]				Mascot
622.3671	622.3768	0.0097	16	914	917	LYKR	Carbamyl (N-term)[0]				Mascot
630.3721	630.3927	0.0206	33	757	761	GWLVR					Mascot
632.3726	632.3942	0.0216	34	534	538	TDLKR					Mascot
633.4003	633.3787	-0.0216	-34	585	589	LLMLK	Oxidation (M)[3]				Mascot
636.4078	636.3929	-0.0149	-23	135	139	FTLKK					Mascot
646.3882	646.3989	0.0107	17	292	296	EKTLR					Mascot
647.3511	647.3797	0.0286	44	829	833	YHSIK					Mascot
648.3827	648.4175	0.0348	54	662	666	FLNVR					Mascot
660.4113	660.3987	-0.0126	-19	585	589	LLMLK	Carbamyl (N-term)[0]				Mascot
662.3984	662.4146	0.0162	24	712	716	QVFLR					Mascot
678.4045	678.4155	0.011	16	727	731	YIRAR					Mascot
680.3627	680.4255	0.0628	92	575	578	YWRR					Mascot
758.4671	758.4481	-0.019	-25	757	762	GWLVRK					Mascot
785.4417	785.4525	0.0108	14	466	471	NFHLVR					Mascot
786.4396	786.4404	0.0008	1	1064	1069	IYSLYK					Mascot
856.5151	856.5213	0.0062	7	859	864	HFLLRK	Carbamyl (N-term)[0]				Mascot
870.4719	870.5401	0.0682	78	741	746	YIEFKK	Carbamyl (N-term)[0]				Mascot
870.4719	870.5401	0.0682	78	741	746	YIEFKK	Carbamyl (N-term)[0]				Mascot

3	Hypothetical protein (Fragment).- Homo sapiens (Human).	Q8NDT6	25	107	100	Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank Result Type
572.3151	572.3632	0.0481	84	720	723	KPER			Carbamyl (N-term)[0]	Mascot
574.3055	574.344	0.0385	67	602	605	ARER			Carbamyl (N-term)[0]	Mascot
576.3099	576.3455	0.0356	62	119	123	TRDGK				Mascot
577.294	577.3192	0.0252	44	78	82	TNQSK				Mascot
579.2806	579.3345	0.0539	93	1	4	ELMK			Carbamyl (N-term)[0], Oxidation (M)[3]	Mascot
580.3453	580.3403	-0.005	-9	109	112	YKVK			Carbamyl (N-term)[0]	Mascot
582.3106	582.3207	0.0101	17	357	360	NHLR			Carbamyl (N-term)[0]	Mascot
584.3151	584.3516	0.0365	62	211	215	QSPPR				Mascot
588.3212	588.36	0.0388	66	610	613	ERQR				Mascot
589.3304	589.3545	0.0241	41	47	51	KSSPK			Carbamyl (N-term)[0]	Mascot
590.2892	590.3466	0.0574	97	606	609	DKER			Carbamyl (N-term)[0]	Mascot
597.3215	597.3434	0.0219	37	239	242	HERR				Mascot
604.3049	604.3631	0.0582	96	571	574	EKER			Carbamyl (N-term)[0]	Mascot
606.3205	606.3562	0.0357	59	724	728	KESSR				Mascot
610.3195	610.3564	0.0369	60	107	110	EKYK			Carbamyl (N-term)[0]	Mascot
615.3572	615.3554	-0.0018	-3	661	665	QSPKR				Mascot
630.3933	630.3927	-0.0006	-1	785	789	AKIQK			Carbamyl (N-term)[0]	Mascot
633.3566	633.3787	0.0221	35	40	45	SSKSPK				Mascot
658.363	658.3967	0.0337	51	661	665	QSPKR			Carbamyl (N-term)[0]	Mascot
660.3423	660.3987	0.0564	85	493	497	VDDRR				Mascot
694.3729	694.4268	0.0539	78	52	58	SSSASKK				Mascot
712.41	712.4024	-0.0076	-11	210	215	KQSPPR				Mascot
786.3853	786.4404	0.0551	70	634	639	EDRNPR				Mascot
807.3525	807.4324	0.0799	99	382	387	NDRMGR			Carbamyl (N-term)[0], Oxidation (M)[4]	Mascot
856.4886	856.5213	0.0327	38	31	38	LSPSPSLR				Mascot
927.4166	927.4974	0.0808	87	309	315	DYSRDTK			Carbamyl (N-term)[0]	Mascot

Gel Idx/Pos	33/B6	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	3

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	AF237621 NID: -	Homo sapiens	AAF60327	17	67	98.281		Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Start Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
670.3267	670.327	0.0003	0	387	392	HGDSVR					Mascot
758.4518	758.4551	0.0033	4	241	246	RVDQLK					Mascot
802.4417	802.4453	0.0036	4	180	185	EREQIK					Mascot
832.4886	832.494	0.0054	6	75	82	SISISVAR					Mascot
973.5312	973.5307	-0.0005	-1	396	403	IEISELNR					Mascot
1033.516	1033.5173	0.0013	1	484	492	TLLEGEESR					Mascot
1057.4844	1057.566	0.0816	77	1	8	MSRQFSSR			Carbamyl (N-term)[0], Oxidation (M)[1]		Mascot
1066.5164	1066.5171	0.0007	1	270	277	YEDEINKR					Mascot
1157.5143	1157.5912	0.0769	66	464	472	DYQELMNTK			Oxidation (M)[6]		Mascot
1179.6005	1179.6021	0.0016	1	377	386	YEELQITAGR					Mascot
1265.6372	1265.6328	-0.0044	-3	278	288	TNAENEFVTIK					Mascot
1308.6429	1308.6587	0.0158	12	278	288	TNAENEFVTIK			Carbamyl (N-term)[0]		Mascot
1332.5195	1332.5164	-0.0031	-2	258	267	NMQDMVEDYR			Oxidation (M)[2,5]		Mascot
1340.6692	1340.6667	-0.0025	-2	365	376	SKAEAESLYQSK					Mascot
1426.6962	1426.6127	-0.0835	-59	186	197	SLNNQFASFIDK			Carbamyl (N-term)[0]		Mascot
1475.7853	1475.7786	-0.0067	-5	200	211	FLEQQNQVLQTK					Mascot
2300.198	2300.1692	-0.0288	-13	344	364	SLDLDSIIAEVKAQNEDIA QK					Mascot
2383.9517	2383.957	0.0053	2	519	549	GGGGGGYGSGGSSYGS GGGSYGSGGGGGGGR					Mascot

2	AF304164 NID: - Homo sapiens	AAG41947	17	66	97.515	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
670.3267	670.327	0.0003	0	387	392	HGDSVR					Mascot
758.4518	758.4551	0.0033	4	241	246	RVDQLK					Mascot
802.4417	802.4453	0.0036	4	180	185	EREQIK					Mascot
832.4886	832.494	0.0054	6	75	82	SISISVAR					Mascot
973.5312	973.5307	-0.0005	-1	396	403	IEISELNR					Mascot
1033.516	1033.5173	0.0013	1	484	492	TLLEGEESR					Mascot
1057.4844	1057.566	0.0816	77	1	8	MSRQFSSR			Carbamyl (N-term)[0], Oxidation (M)[1]		Mascot
1065.5211	1065.509	-0.0121	-11	356	364	AQYEDIAQK					Mascot
1066.5164	1066.5171	0.0007	1	270	277	YEDEINKR					Mascot
1157.5143	1157.5912	0.0769	66	464	472	DYQELMNTK			Oxidation (M)[6]		Mascot
1179.6005	1179.6021	0.0016	1	377	386	YEELQITAGR					Mascot
1265.6372	1265.6328	-0.0044	-3	278	288	TNAENEFVTIK					Mascot
1308.6429	1308.6587	0.0158	12	278	288	TNAENEFVTIK			Carbamyl (N-term)[0]		Mascot
1332.5195	1332.5164	-0.0031	-2	258	267	NMQDMVEDYR			Oxidation (M)[2,5]		Mascot
1340.6692	1340.6667	-0.0025	-2	365	376	SKAEAESLYQSK					Mascot
1426.6962	1426.6127	-0.0835	-59	186	197	SLNNQFASFIDK			Carbamyl (N-term)[0]		Mascot
1475.7853	1475.7786	-0.0067	-5	200	211	FLEQQNQVLQTK					Mascot
2383.9517	2383.957	0.0053	2	519	549	GGGGGGYGSGGSSYGS GGGSYGSGGGGGGGR					Mascot

3	HUMKTEP2A NID: - Homo sapiens	AAA36153	15	62	94.438	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type

670.3267	70.327	0.0003	0	236	241	HGDSVR		Mascot
758.4518	758.4551	0.0033	4	90	95	RVDQLK		Mascot
802.4417	802.4453	0.0036	4	29	34	EREQIK		Mascot
832.4821	832.494	0.0119	14	253	258	MIQRLR	Oxidation (M)[1]	Mascot
973.5312	973.5307	-0.0005	-1	245	252	IEISELNR		Mascot
1033.516	1033.5173	0.0013	1	333	341	TLLEGEESR		Mascot
1065.5211	1065.509	-0.0121	-11	205	213	AQYEDIAQK		Mascot
1066.5164	1066.5171	0.0007	1	119	126	YEDEINKR		Mascot
1179.6005	1179.6021	0.0016	1	226	235	YEELQITAGR		Mascot
1265.6372	1265.6328	-0.0044	-3	127	137	TNAENEFVTIK		Mascot
1308.6429	1308.6587	0.0158	12	127	137	TNAENEFVTIK	Carbamyl (N-term)[0]	Mascot
1332.5195	1332.5164	-0.0031	-2	107	116	NMQDMVEDYR	Oxidation (M)[2,5]	Mascot
1340.6692	1340.6667	-0.0025	-2	214	225	SKAEAESLYQSK		Mascot
1426.6962	1426.6127	-0.0835	-59	35	46	SLNNQFASFIDK	Carbamyl (N-term)[0]	Mascot
1475.7489	1475.7786	0.0297	20	61	72	WELLQQVDTSTR		Mascot
1493.7781	1493.7363	-0.0418	-28	49	60	FMEQQNKVLQTK		Mascot

Gel Idx/Pos	34/B7	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	2

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	AY099890 NID:	- Homo sapiens	AAM44119	30	146	100		Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Start Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3627	572.3243	-0.0384	-67	443	446	QIRR					Mascot
578.333	578.322	-0.011	-19	542	545	IMKK			Carbamyl (N-term)[0], Oxidation (M)[2]		Mascot
579.3613	579.3526	-0.0087	-15	56	59	LYKR					Mascot
586.3783	586.3209	-0.0574	-98	729	733	GVRVR					Mascot
589.3304	589.3125	-0.0179	-30	230	234	NATIK			Carbamyl (N-term)[0]		Mascot
590.3442	590.3206	-0.0236	-40	583	587	GMKVR					Mascot
592.3314	592.32	-0.0114	-19	1	4	RAFR			Carbamyl (N-term)[0]		Mascot
596.3627	596.3249	-0.0378	-63	807	810	RHLK			Carbamyl (N-term)[0]		Mascot
600.3939	600.3496	-0.0443	-74	656	660	GIRVR					Mascot
602.362	602.3259	-0.0361	-60	77	81	GKIER					Mascot
610.3195	610.3375	0.018	29	218	222	AYVSK			Carbamyl (N-term)[0]		Mascot
611.3008	611.3193	0.0185	30	146	149	HQQR			Carbamyl (N-term)[0]		Mascot
615.3685	615.3622	-0.0063	-10	443	446	QIRR			Carbamyl (N-term)[0]		Mascot
621.3177	621.3274	0.0097	16	875	878	WMIR			Oxidation (M)[2]		Mascot
622.3671	622.3284	-0.0387	-62	364	368	GYKVR					Mascot
631.3522	631.3422	-0.01	-16	768	772	TVQQR					Mascot
634.3922	634.3792	-0.013	-20	129	133	FLNLK					Mascot
637.3416	637.3474	0.0058	9	387	391	GYNKR					Mascot
648.3286	648.373	0.0444	68	875	878	WMIR			Carbamyl (N-term)[0]		Mascot

	650.3871	650.3904	0.0033	5	832	836	FLSLK		Carbamyl (N-term)[0]		Mascot
	674.358	674.3748	0.0168	25	768	772	TVQQR		Carbamyl (N-term)[0]		Mascot
	676.381	676.3788	-0.0022	-3	460	465	MAKAQK				Mascot
	686.4307	686.3846	-0.0461	-67	267	272	IAAQKR				Mascot
	694.3375	694.3929	0.0554	80	825	829	TLMMR		Carbamyl (N-term)[0]		Mascot
	704.3474	704.3804	0.033	47	483	488	AWTAGR		Carbamyl (N-term)[0]		Mascot
	706.3665	706.398	0.0315	45	241	245	MKQTR		Carbamyl (N-term)[0]		Mascot
	733.4177	733.4103	-0.0074	-10	875	879	WMIRK				Mascot
	785.39	785.45	0.06	76	413	418	TLHDTR		Carbamyl (N-term)[0]		Mascot
	809.4152	809.3856	-0.0296	-37	82	87	TNYLQK		Carbamyl (N-term)[0]		Mascot
	856.4457	856.5209	0.0752	88	27	32	MHKELR		Carbamyl (N-term)[0]		Mascot
	864.5189	864.4921	-0.0268	-31	223	229	KEFLSLK				Mascot
	868.4788	868.529	0.0502	58	5	10	EWHLRK				Mascot
	923.4403	923.5266	0.0863	93	684	690	ISYHTMR		Oxidation (M)[6]		Mascot

2	AF509326 NID: - Homo sapiens	AAN40011	49	130	100	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.2973	572.3243	0.027	47	2350	2353	LHMR			Oxidation (M)[3]		Mascot
576.3099	576.3246	0.0147	26	2730	2733	TERK			Carbamyl (N-term)[0]		Mascot
577.2576	577.2831	0.0255	44	291	295	GENSK			Carbamyl (N-term)[0]		Mascot
578.3296	578.322	-0.0076	-13	3160	3163	FIQK			Carbamyl (N-term)[0]		Mascot
579.35	579.3526	0.0026	4	3417	3420	ILYK			Carbamyl (N-term)[0]		Mascot
580.3201	580.3148	-0.0053	-9	3042	3046	GYKGR					Mascot
581.3041	581.3121	0.008	14	755	759	AYTAR					Mascot
583.2908	583.306	0.0152	26	2951	2954	YLCK			Carbamidomethyl (C)[3]		Mascot
586.3671	586.3209	-0.0462	-79	1329	1333	VLAQR					Mascot

589.3304	589.3125	-0.0179	-30	1680	1684	NATIK	Carbamyl (N-term)[0]	Mascot
590.3296	590.3206	-0.009	-15	695	698	WKEK		Mascot
592.3235	592.32	-0.0035	-6	2397	2401	GMKTR		Mascot
594.3609	594.3336	-0.0273	-46	1280	1283	YKLK	Carbamyl (N-term)[0]	Mascot
596.3627	596.3249	-0.0378	-63	2402	2405	RHLK	Carbamyl (N-term)[0]	Mascot
600.3099	600.3496	0.0397	66	467	471	QNNPK		Mascot
602.362	602.3259	-0.0361	-60	1527	1531	GKIER		Mascot
610.3195	610.3375	0.018	29	1668	1672	AYVSK	Carbamyl (N-term)[0]	Mascot
611.3008	611.3193	0.0185	30	1596	1599	HQQR	Carbamyl (N-term)[0]	Mascot
614.362	614.3483	-0.0137	-22	740	744	ISVPR	Carbamyl (N-term)[0]	Mascot
615.3685	615.3622	-0.0063	-10	1893	1896	QIRR	Carbamyl (N-term)[0]	Mascot
616.3525	616.3519	-0.0006	-1	115	119	EGRVR		Mascot
621.3177	621.3274	0.0097	16	2325	2328	WMIR	Oxidation (M)[2]	Mascot
622.3671	622.3284	-0.0387	-62	1814	1818	GYKVR		Mascot
629.3729	629.3206	-0.0523	-83	1329	1333	VLAQR	Carbamyl (N-term)[0]	Mascot
631.3521	631.3422	-0.0099	-16	3065	3070	AREAGK		Mascot
634.3705	634.3792	0.0087	14	2828	2832	MVTRK		Mascot
637.3416	637.3474	0.0058	9	1837	1841	GYNKR		Mascot
646.377	646.3539	-0.0231	-36	1341	1345	EKLEK		Mascot
648.3827	648.373	-0.0097	-15	2997	3001	FLNVR		Mascot
650.3871	650.3904	0.0033	5	2427	2431	FISLK	Carbamyl (N-term)[0]	Mascot
660.3575	660.3689	0.0114	17	1434	1437	WKQR	Carbamyl (N-term)[0]	Mascot
674.358	674.3748	0.0168	25	2218	2222	TVQQR	Carbamyl (N-term)[0]	Mascot
676.381	676.3788	-0.0022	-3	2955	2960	VKAACK	Carbamidomethyl (C)[5]	Mascot
678.4045	678.3821	-0.0224	-33	3062	3066	YIRAR		Mascot
686.4307	686.3846	-0.0461	-67	2519	2524	AAKLQR		Mascot
694.363	694.3929	0.0299	43	2587	2591	YRANK	Carbamyl (N-term)[0]	Mascot
703.4097	703.3747	-0.035	-50	3393	3398	SKVVDR		Mascot

	704.3474	704.3804	0.033	47	1933	1938	AWTAGR	Carbamyl (N-term)[0]		Mascot
	706.3665	706.398	0.0315	45	1691	1695	MKQTR	Carbamyl (N-term)[0]		Mascot
	733.4177	733.4103	-0.0074	-10	2325	2329	WMIRK			Mascot
	760.4563	760.4073	-0.049	-64	1282	1287	LKTDLK	Carbamyl (N-term)[0]		Mascot
	785.4417	785.45	0.0083	11	1216	1221	NFHLVR			Mascot
	809.4152	809.3856	-0.0296	-37	1532	1537	TNYLQK	Carbamyl (N-term)[0]		Mascot
	826.4165	826.4605	0.044	53	1289	1294	HQEREK			Mascot
	856.5151	856.5209	0.0058	7	3194	3199	HFLLRK	Carbamyl (N-term)[0]		Mascot
	864.5189	864.4921	-0.0268	-31	1673	1679	KEFLSLK			Mascot
	868.4788	868.529	0.0502	58	1455	1460	EWHLRK			Mascot
	870.4719	870.5416	0.0697	80	3076	3081	YIEFKK	Carbamyl (N-term)[0]		Mascot
	870.4719	870.5416	0.0697	80	3076	3081	YIEFKK	Carbamyl (N-term)[0]		Mascot
	923.4832	923.5266	0.0434	47	3245	3251	EENKLYK			Mascot
	1057.5901	1057.5675	-0.0226	-21	2895	2903	AFLSAKHQR			Mascot

3	GAC-1.- Homo sapiens (Human).	Q9H231	37	100	100	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
570.2994	570.3154	0.016	28	1033	1036	HKDK			Carbamyl (N-term)[0]		Mascot
576.2987	576.3246	0.0259	45	1056	1061	SSPASK					Mascot
578.278	578.322	0.044	76	46	50	SDVSK			Carbamyl (N-term)[0]		Mascot
580.2759	580.3148	0.0389	67	642	645	KMDK			Carbamyl (N-term)[0], Oxidation (M)[2]		Mascot
581.3517	581.3121	-0.0396	-68	379	382	HILR			Carbamyl (N-term)[0]		Mascot
582.3245	582.3199	-0.0046	-8	676	679	YKTK			Carbamyl (N-term)[0]		Mascot
590.3442	590.3206	-0.0236	-40	1911	1914	MKLR			Carbamyl (N-term)[0]		Mascot
591.3096	591.3163	0.0067	11	946	950	SEKGK			Carbamyl (N-term)[0]		Mascot
592.33	592.32	-0.01	-17	735	739	STKEK					Mascot

596.3401	596.3249	-0.0152	-25	410	414	SFKSK		Mascot
602.278	602.3259	0.0479	80	104	108	EGPEK	Carbamyl (N-term)[0]	Mascot
610.3195	610.3375	0.018	29	674	677	EKYK	Carbamyl (N-term)[0]	Mascot
615.3572	615.3622	0.005	8	416	420	QKPSR		Mascot
616.3525	616.3519	-0.0006	-1	1108	1111	ERLR	Carbamyl (N-term)[0]	Mascot
622.2977	622.3284	0.0307	49	836	839	MERK	Carbamyl (N-term)[0], Oxidation (M)[1]	Mascot
627.2984	627.3132	0.0148	24	99	103	TYSEK		Mascot
629.3616	629.3206	-0.041	-65	1153	1157	EKQPK		Mascot
631.3885	631.3422	-0.0463	-73	825	829	RSQIK		Mascot
634.3592	634.3792	0.02	32	1083	1087	MLSLK	Carbamyl (N-term)[0]	Mascot
646.3518	646.3539	0.0021	3	176	180	DKVNK	Carbamyl (N-term)[0]	Mascot
650.3871	650.3904	0.0033	5	1997	2001	FDKLK		Mascot
652.3664	652.3762	0.0098	15	839	843	KTFEK		Mascot
660.3675	660.3689	0.0014	2	890	895	NTAAIK	Carbamyl (N-term)[0]	Mascot
674.3831	674.3748	-0.0083	-12	1846	1850	IEEKR		Mascot
676.326	676.3788	0.0528	78	749	753	EERDK		Mascot
678.3239	678.3821	0.0582	86	905	909	EKMDR		Mascot
684.3351	684.349	0.0139	20	698	702	ENFFK		Mascot
703.4097	703.3747	-0.035	-50	620	625	SNAKIK	Carbamyl (N-term)[0]	Mascot
704.3461	704.3804	0.0343	49	668	673	EIEGEK		Mascot
733.4202	733.4103	-0.0099	-13	1137	1142	ELTRSK		Mascot
760.3835	760.4073	0.0238	31	484	489	ENQELK		Mascot
785.4338	785.45	0.0162	21	929	934	HLMEKK		Mascot
809.4008	809.3856	-0.0152	-19	2002	2007	TCLLMR	Carbamidomethyl (C)[2], Oxidation (M)[5]	Mascot
826.4053	826.4605	0.0552	67	977	982	HIQEEK	Carbamyl (N-term)[0]	Mascot
856.4635	856.5209	0.0574	67	1281	1287	LSTSHLR	Carbamyl (N-term)[0]	Mascot
923.4502	923.5266	0.0764	83	46	53	SDVSKEMK		Mascot
1057.5385	1057.5675	0.029	27	1155	1163	QPKDAVSNR	Carbamyl (N-term)[0]	Mascot

Gel Idx/Pos	35/B8	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	3

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	Hypothetical protein FLJ20291.- Homo sapiens (Human).		Q9NXE8	21	125	100		Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
570.3358	570.3315	-0.0043	-8	270	274	HASKK					Mascot
572.3303	572.3458	0.0155	27	386	389	FIHR					Mascot
577.2463	577.245	-0.0013	-2	146	149	EEEK			Carbamyl (N-term)[0]		Mascot
579.2443	579.2448	0.0005	1	100	103	MEEK			Carbamyl (N-term)[0]		Mascot
582.2704	582.2956	0.0252	43	421	424	NFMK			Carbamyl (N-term)[0]		Mascot
598.3307	598.3511	0.0204	34	301	305	LHNSK					Mascot
613.3416	613.3566	0.015	24	270	274	HASKK			Carbamyl (N-term)[0]		Mascot
615.3361	615.3824	0.0463	75	386	389	FIHR			Carbamyl (N-term)[0]		Mascot
686.3831	686.3721	-0.011	-16	316	321	SPSPKK			Carbamyl (N-term)[0]		Mascot
708.298	708.3356	0.0376	53	51	55	EEMQR			Oxidation (M)[3]		Mascot
773.4151	773.3471	-0.068	-88	2	9	GGGDLNLK					Mascot
802.4417	802.4377	-0.004	-5	373	378	EQRLEK					Mascot
822.4468	822.4609	0.0141	17	321	326	KEVYQR					Mascot
904.4556	904.4505	-0.0051	-6	1	9	MGGGDLNLK					Mascot
906.4387	906.4487	0.01	11	275	282	STREAGSR			Carbamyl (N-term)[0]		Mascot
980.4175	980.4435	0.026	27	346	353	QEMMENAK					Mascot
989.4897	989.5359	0.0462	47	336	343	LSAEELER			Carbamyl (N-term)[0]		Mascot
994.4839	994.5203	0.0364	37	56	64	YAEDVGAVK			Carbamyl (N-term)[0]		Mascot

	994.4839	994.5203	0.0364	37	56	64	YAEDVGAVK			Carbamyl (N-term)[0]		Mascot
	1032.5507	1032.567	0.0163	16	1	10	MGGGDLNLKK					Mascot
	1066.496	1066.5188	0.0228	21	251	259	GHGMKNHSR			Carbamyl (N-term)[0]		Mascot
	1106.5762	1106.5316	-0.0446	-40	165	173	ELLQMSLEK			Oxidation (M)[5]		Mascot
	1179.5245	1179.6018	0.0773	66	345	353	RQEMMENAK			Carbamyl (N-term)[0]		Mascot
	1314.7528	1314.6664	-0.0864	-66	134	143	IREDPLFIIR			Carbamyl (N-term)[0]		Mascot

2	autoantigen, 64K - human	S18732	21	99	99.999	Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3151	572.3458	0.0307	54	96	99	EPKR			Carbamyl (N-term)[0]		Mascot
577.2463	577.245	-0.0013	-2	160	163	EEEK			Carbamyl (N-term)[0]		Mascot
579.2443	579.2448	0.0005	1	181	184	EEMK			Carbamyl (N-term)[0]		Mascot
616.3412	616.3807	0.0395	64	495	499	NSPKK			Carbamyl (N-term)[0]		Mascot
621.3024	621.338	0.0356	57	533	537	KMGDK			Carbamyl (N-term)[0]		Mascot
677.31	677.3287	0.0187	28	238	242	KDDEK			Carbamyl (N-term)[0]		Mascot
708.3345	708.3356	0.0011	2	180	184	REEMK			Oxidation (M)[4]		Mascot
717.3525	717.4173	0.0648	90	456	461	QAQEAK			Carbamyl (N-term)[0]		Mascot
758.4518	758.4495	-0.0023	-3	135	141	VRAAVDK					Mascot
775.3693	775.4006	0.0313	40	143	149	EAGKDGR			Carbamyl (N-term)[0]		Mascot
807.3777	807.4021	0.0244	30	442	447	NMDKQR			Oxidation (M)[2]		Mascot
809.3457	809.4164	0.0707	87	214	221	AGGNTDMK			Oxidation (M)[7]		Mascot
856.4774	856.5241	0.0467	55	466	472	DLLEVPK			Carbamyl (N-term)[0]		Mascot
906.3911	906.4487	0.0576	64	109	116	DRDESGGK			Carbamyl (N-term)[0]		Mascot
967.4591	967.4321	-0.027	-28	30	37	QSTGVYNR			Carbamyl (N-term)[0]		Mascot
980.4465	980.4435	-0.003	-3	214	222	AGGNTDMKK			Carbamyl (N-term)[0], Oxidation (M)[7]		Mascot
994.5316	994.5203	-0.0113	-11	245	252	KNEPLHEK					Mascot

	994.5316	994.5203	-0.0113	-11	245	252	KNEPLHEK					Mascot
	1018.4911	1018.4843	-0.0068	-7	68	76	TDAKNGQER					Mascot
	1032.5181	1032.567	0.0489	47	165	174	GGDRNTGLSR					Mascot
	1034.5663	1034.5308	-0.0355	-34	433	441	MTVTNLLSR					Mascot
	1144.6069	1144.5175	-0.0894	-78	524	533	NSLSPATQRK		Carbamyl (N-term)[0]			Mascot

3	XAP-5-like protein (DNA segment ON chromosome 6(UNIQUE) 2654 expressed sequence).- Homo sapiens (Hu	Q9Y247	16	92	99.994	Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
615.3936	615.3824	-0.0112	-18	87	91	QLAKR					Mascot
700.41	700.4023	-0.0077	-11	216	221	ALQGLR			Carbamyl (N-term)[0]		Mascot
712.33	712.3431	0.0131	18	296	300	SWYEK					Mascot
758.4267	758.4495	0.0228	30	138	144	RAGNLGK			Carbamyl (N-term)[0]		Mascot
775.4056	775.4006	-0.005	-6	43	48	SQVDKR			Carbamyl (N-term)[0]		Mascot
785.4991	785.4529	-0.0462	-59	216	222	ALQGLRK					Mascot
786.3376	786.4103	0.0727	92	285	291	DESHAGK			Carbamyl (N-term)[0]		Mascot
856.5073	856.5241	0.0168	20	14	20	AMHLLKK			Oxidation (M)[2]		Mascot
870.458	870.5425	0.0845	97	303	309	HIFPASR			Carbamyl (N-term)[0]		Mascot
989.4435	989.5359	0.0924	93	171	177	QEWEAQR			Carbamyl (N-term)[0]		Mascot
1034.5629	1034.5308	-0.0321	-31	206	214	GNTVQQFLK					Mascot
1066.5085	1066.5188	0.0103	10	276	284	LLSDATMEK			Carbamyl (N-term)[0], Oxidation (M)[7]		Mascot
1112.5959	1112.5317	-0.0642	-58	301	309	NKHIFPASR			Carbamyl (N-term)[0]		Mascot
1128.5289	1128.5293	0.0004	0	1	9	MAQYKGTMR			Carbamyl (N-term)[0]		Mascot
1144.5238	1144.5175	-0.0063	-6	1	9	MAQYKGTMR			Carbamyl (N-term)[0], Oxidation (M)[1]		Mascot
1165.5524	1165.5884	0.036	31	310	318	WEAYDPEKK					Mascot
1179.6521	1179.6018	-0.0503	-43	292	300	VVLRSWYEK					Mascot

Gel Idx/Pos	36/B9	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	3

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	alpha-1-acid glycoprotein 2 precursor - human		OMHU2	11	138	100	68	Mascot	100

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Start Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
581.2678	581.2604	-0.0074	-13	109	113	YEGGR					Mascot
696.3311	696.3339	0.0028	4	82	86	EYQTR					Mascot
775.3654	775.3696	0.0042	5	183	188	CEPLEK			Carbamidomethyl (C)[1]		Mascot
994.5203	994.5211	0.0008	1	74	81	TEDTIFLR					Mascot
994.5203	994.5211	0.0008	1	74	81	TEDTIFLR	68	100			Mascot
1018.4873	1018.4857	-0.0016	-2	181	188	DKCEPLEK			Carbamidomethyl (C)[3]		Mascot
1144.4979	1144.5146	0.0167	15	171	179	SDVMYTDWK					Mascot
1160.5887	1160.5548	-0.0339	-29	43	51	WFYIASAFR					Mascot
1234.7054	1234.7079	0.0025	2	114	123	EHVAHLLFLR					Mascot
1272.5929	1272.6096	0.0167	13	171	180	SDVMYTDWKK					Mascot
1461.6566	1461.6532	-0.0034	-2	127	138	TLMFGSYLDDEK			Carbamyl (N-term)[0]		Mascot
2112.9729	2112.9697	-0.0032	-2	154	170	EQLGEFYEALDCLCIPR			Carbamidomethyl (C)[12,14]		Mascot

2	BC026238 NID: - Homo sapiens	AAH26238	9	120	100	68	Mascot	100

Protein Group

HSA1GPA1 NID: - Homo sapiens	CAA29229
alpha-1-acid glycoprotein 1 precursor [validated] - human	OMHU1

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
696.3311	696.3339	0.0028	4	82	86	EYQTR					Mascot
775.3654	775.3696	0.0042	5	183	188	CEPLEK			Carbamidomethyl (C)[1]		Mascot
994.5203	994.5211	0.0008	1	74	81	TEDTIFLR					Mascot
994.5203	994.5211	0.0008	1	74	81	TEDTIFLR	68	100			Mascot
1018.4873	1018.4857	-0.0016	-2	181	188	DKCEPLEK			Carbamidomethyl (C)[3]		Mascot
1112.5259	1112.5226	-0.0033	-3	171	179	SDVVYTDWK					Mascot
1160.5887	1160.5548	-0.0339	-29	43	51	WFYIASAFR					Mascot
1445.6617	1445.6577	-0.004	-3	127	138	TYMLAFDVNDEK					Mascot
1461.6566	1461.6532	-0.0034	-2	127	138	TYMLAFDVNDEK			Oxidation (M)[3]		Mascot
1742.8054	1742.8112	0.0058	3	154	167	EQLGEFYEALDCLR			Carbamidomethyl (C)[12]		Mascot
1752.9543	1752.9724	0.0181	10	109	123	YVGGQEHFAHLLILR					Mascot

3	myosin heavy chain VA - human (fragment)	A53016	25	112	100	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3878	572.3448	-0.043	-75	221	224	KILR			Carbamyl (N-term)[0]		Mascot
579.3613	579.3304	-0.0309	-53	196	199	YKIR					Mascot
594.2994	594.3301	0.0307	52	165	169	GYQAR					Mascot
664.3447	664.3784	0.0337	51	87	91	KQTCK			Carbamidomethyl (C)[4]		Mascot
696.3199	696.3339	0.014	20	454	458	YDDLK			Carbamyl (N-term)[0]		Mascot
775.358	775.3696	0.0116	15	298	303	VDEQNK			Carbamyl (N-term)[0]		Mascot
854.4882	854.4462	-0.042	-49	109	114	TKIFFR			Carbamyl (N-term)[0]		Mascot
868.4709	868.5273	0.0564	65	73	78	YRVLMK			Carbamyl (N-term)[0], Oxidation (M)[5]		Mascot
870.5519	870.5424	-0.0095	-11	228	234	AVIIQKR			Carbamyl (N-term)[0]		Mascot
948.4897	948.4904	0.0007	1	55	63	ISAAGFPSR			Carbamyl (N-term)[0]		Mascot

961.5247	961.4737	-0.051	-53	154	161	KAAITMQR	Carbamyl (N-term)[0]	Mascot
973.604	973.5213	-0.0827	-85	178	186	TKAATIIQK		Mascot
1016.6098	1016.5141	-0.0957	-94	178	186	TKAATIIQK	Carbamyl (N-term)[0]	Mascot
1018.5051	1018.4857	-0.0194	-19	524	531	VTELEQEK	Carbamyl (N-term)[0]	Mascot
1030.488	1030.495	0.007	7	31	38	FPFTFDEK		Mascot
1057.6266	1057.5657	-0.0609	-58	139	146	TIRGWLLR	Carbamyl (N-term)[0]	Mascot
1112.5881	1112.5226	-0.0655	-59	282	290	KLHIGMENK	Carbamyl (N-term)[0]	Mascot
1116.5255	1116.5179	-0.0076	-7	165	173	GYQARCYAK	Carbamidomethyl (C)[6]	Mascot
1124.5807	1124.515	-0.0657	-58	398	406	QEKEALNHR		Mascot
1126.512	1126.5227	0.0107	9	413	421	EMTETMEKK		Mascot
1128.583	1128.5234	-0.0596	-53	282	290	KLHIGMENK	Carbamyl (N-term)[0], Oxidation (M)[6]	Mascot
1148.5542	1148.616	0.0618	54	360	368	DLEQTRSEK	Carbamyl (N-term)[0]	Mascot
1176.5314	1176.5654	0.034	29	532	540	QVMQDELDR	Carbamyl (N-term)[0]	Mascot
1179.6005	1179.5985	-0.002	-2	450	458	LQERYDDLK		Mascot
1192.5262	1192.4862	-0.04	-34	532	540	QVMQDELDR	Carbamyl (N-term)[0], Oxidation (M)[3]	Mascot
1234.7155	1234.7079	-0.0076	-6	201	211	AATIVLQSYLR		Mascot
1740.8142	1740.8396	0.0254	15	407	420	IVQQAKEMTETMEK	Carbamyl (N-term)[0], Oxidation (M)[8,12]	Mascot
1742.9041	1742.8112	-0.0929	-53	283	296	LHIGMENKIMQLQR	Oxidation (M)[5,10]	Mascot

Gel Idx/Pos	37/B10	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	2

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	AF509326 NID: -	Homo sapiens	AAN40011	46	114	100		Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3627	572.364	0.0013	2	1893	1896	QIRR					Mascot
573.3467	573.3447	-0.002	-3	2754	2757	VRQK			Carbamyl (N-term)[0]		Mascot
574.3671	574.3564	-0.0107	-19	1960	1964	GKTLR					Mascot
576.3099	576.3544	0.0445	77	2730	2733	TERK			Carbamyl (N-term)[0]		Mascot
578.333	578.3391	0.0061	11	1992	1995	IMKK			Carbamyl (N-term)[0], Oxidation (M)[2]		Mascot
579.35	579.3429	-0.0071	-12	3417	3420	ILYK			Carbamyl (N-term)[0]		Mascot
580.3201	580.3598	0.0397	68	2946	2949	YRAK			Carbamyl (N-term)[0]		Mascot
581.3041	581.3392	0.0351	60	755	759	AYTAR					Mascot
584.3151	584.373	0.0579	99	2552	2555	EKHK			Carbamyl (N-term)[0]		Mascot
586.3671	586.3617	-0.0054	-9	1329	1333	VLAQR					Mascot
588.3575	588.3728	0.0153	26	2184	2187	RTLR			Carbamyl (N-term)[0]		Mascot
590.3442	590.3549	0.0107	18	2751	2755	GMKVR					Mascot
592.3453	592.3472	0.0019	3	2922	2926	GFIQK					Mascot
594.3609	594.3525	-0.0084	-14	1280	1283	YKLK			Carbamyl (N-term)[0]		Mascot
596.3627	596.3441	-0.0186	-31	2402	2405	RHLK			Carbamyl (N-term)[0]		Mascot
599.3082	599.3633	0.0551	92	2350	2353	LHMR			Carbamyl (N-term)[0]		Mascot
600.3939	600.3804	-0.0135	-22	2106	2110	GIRVR					Mascot
602.366	602.3677	0.0017	3	1047	1050	LLWK			Carbamyl (N-term)[0]		Mascot

603.3824	603.3754	-0.007	-12	2095	2099	KTAIK	Carbamyl (N-term)[0]	Mascot
606.3721	606.3706	-0.0015	-2	2678	2682	GFKVR		Mascot
608.3184	608.372	0.0536	88	2397	2401	GMKTR	Oxidation (M)[2]	Mascot
610.3558	610.3638	0.008	13	2056	2060	AYKTK		Mascot
615.3685	615.3758	0.0073	12	1893	1896	QIRR	Carbamyl (N-term)[0]	Mascot
616.3525	616.3807	0.0282	46	115	119	EGRVR		Mascot
618.3456	618.3661	0.0205	33	2833	2837	LETQK		Mascot
629.3729	629.3663	-0.0066	-10	1329	1333	VLAQR	Carbamyl (N-term)[0]	Mascot
632.3726	632.395	0.0224	35	1284	1288	TDLKR		Mascot
633.3929	633.3716	-0.0213	-34	611	616	TSAVKK		Mascot
634.3956	634.396	0.0004	1	2471	2475	LMVKK	Oxidation (M)[2]	Mascot
636.4078	636.4071	-0.0007	-1	885	889	FTLKK		Mascot
642.3504	642.3972	0.0468	73	2701	2705	MHRAK		Mascot
646.4069	646.4128	0.0059	9	2470	2474	RLMVK		Mascot
650.3984	650.4087	0.0103	16	2354	2358	YRALK		Mascot
658.4246	658.4147	-0.0099	-15	1716	1721	KIAAQK		Mascot
662.4017	662.4238	0.0221	33	2470	2474	RLMVK	Oxidation (M)[3]	Mascot
674.3943	674.412	0.0177	26	2992	2996	LERTR		Mascot
676.4061	676.4161	0.01	15	1335	1339	LLMLK	Carbamyl (N-term)[0], Oxidation (M)[3]	Mascot
678.4045	678.4203	0.0158	23	3062	3066	YIRAR		Mascot
720.3708	720.4262	0.0554	77	1439	1444	MQSQVK		Mascot
779.4297	779.4075	-0.0222	-28	2089	2094	EYLNLK		Mascot
785.4417	785.4509	0.0092	12	1216	1221	NFHLVR		Mascot
802.4417	802.4463	0.0046	6	431	437	KSEVSPR		Mascot
854.4631	854.513	0.0499	58	799	804	DRHLWK		Mascot
856.5151	856.525	0.0099	12	3194	3199	HFLLRK	Carbamyl (N-term)[0]	Mascot
868.4788	868.5259	0.0471	54	1455	1460	EWHLRK		Mascot
870.4719	870.5427	0.0708	81	3076	3081	YIEFKK	Carbamyl (N-term)[0]	Mascot
870.4719	870.5427	0.0708	81	3076	3081	YIEFKK	Carbamyl (N-term)[0]	Mascot

	902.4941	902.4745	-0.0196	-22	619	626	NVTTPISK			Carbamyl (N-term)[0]			Mascot
	1057.5425	1057.5658	0.0233	22	2596	2603	VFQHNELK			Carbamyl (N-term)[0]			Mascot
	1179.5867	1179.6064	0.0197	17	1769	1776	QYYLKMYK			Carbamyl (N-term)[0]			Mascot
2	Similar to ribosomal protein L35.- Homo sapiens (Human).							Q96QJ7	13	111	100	Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
573.3467	573.3447	-0.002	-3	94	97	RLNK			Carbamyl (N-term)[0]		Mascot
574.3671	574.3564	-0.0107	-19	52	56	KSIAR					Mascot
576.3034	576.3544	0.051	88	90	93	AMRR			Carbamyl (N-term)[0]		Mascot
579.3248	579.3429	0.0181	31	119	123	YAVRA					Mascot
588.3463	588.3728	0.0265	45	8	12	DLRGK					Mascot
590.3694	590.3549	-0.0145	-25	1	5	MAKIK					Mascot
592.2983	592.3472	0.0489	83	90	93	AMRR			Carbamyl (N-term)[0], Oxidation (M)[2]		Mascot
602.3369	602.3677	0.0308	51	107	110	QQRK			Carbamyl (N-term)[0]		Mascot
606.3643	606.3706	0.0063	10	1	5	MAKIK			Oxidation (M)[1]		Mascot
616.414	616.3807	-0.0333	-54	44	48	LSKIR					Mascot
633.3752	633.3716	-0.0036	-6	1	5	MAKIK			Carbamyl (N-term)[0]		Mascot
634.3453	634.396	0.0507	80	88	92	TRAMR					Mascot
636.3827	636.4071	0.0244	38	118	122	KYAVR					Mascot
642.3609	642.3972	0.0363	57	72	76	FYKGK					Mascot
674.3719	674.412	0.0401	59	15	19	EELLK			Carbamyl (N-term)[0]		Mascot
802.4668	802.4463	-0.0205	-26	14	19	KEELLK			Carbamyl (N-term)[0]		Mascot

3	ribosomal protein L35 - human	G01477	13	111	100	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
573.3467	573.3447	-0.002	-3	94	97	RLNK			Carbamyl (N-term)[0]		Mascot
574.3671	574.3564	-0.0107	-19	52	56	KSIAR					Mascot
576.3034	576.3544	0.051	88	90	93	AMRR			Carbamyl (N-term)[0]		Mascot
588.3463	588.3728	0.0265	45	8	12	DLRGK					Mascot
590.3694	590.3549	-0.0145	-25	1	5	MAKIK					Mascot
592.2983	592.3472	0.0489	83	90	93	AMRR			Carbamyl (N-term)[0], Oxidation (M)[2]		Mascot
594.3245	594.3525	0.028	47	119	123	YAVKA			Carbamyl (N-term)[0]		Mascot
602.3369	602.3677	0.0308	51	107	110	QQRK			Carbamyl (N-term)[0]		Mascot
606.3643	606.3706	0.0063	10	1	5	MAKIK			Oxidation (M)[1]		Mascot
608.3765	608.372	-0.0045	-7	118	122	KYAVK					Mascot
616.414	616.3807	-0.0333	-54	44	48	LSKIR					Mascot
633.3752	633.3716	-0.0036	-6	1	5	MAKIK			Carbamyl (N-term)[0]		Mascot
634.3453	634.396	0.0507	80	88	92	TRAMR					Mascot
642.3609	642.3972	0.0363	57	72	76	FYKGK					Mascot
674.3719	674.412	0.0401	59	15	19	EELLK			Carbamyl (N-term)[0]		Mascot
802.4668	802.4463	-0.0205	-26	14	19	KEELLK			Carbamyl (N-term)[0]		Mascot

Gel Idx/Pos	38/B11	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	2

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	haptoglobin precursor, allele 1 [validated] - human		HPHU1	15	149	100	64	Mascot	100

Protein Group

HSHP1G1 NID: - Homo sapiens — CAA25926

HUAC004682 NID: - Homo sapiens — AAC27432

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
760.3987	760.3983	-0.0004	-1	233	238	FTDHLK					Mascot
809.3787	809.384	0.0053	7	212	218	DYAEVGR					Mascot
856.4675	856.5224	0.0549	64	54	59	NYYKLR					Mascot
858.493	858.4936	0.0006	1	170	176	QLVEIEK					Mascot
920.4624	920.4621	-0.0003	0	112	119	GSFPWQAK					Mascot
923.5308	923.5319	0.0011	1	103	111	ILGGHLDAK					Mascot
923.5308	923.5319	0.0011	1	103	111	ILGGHLDAK	64	100			Mascot
980.4948	980.4982	0.0034	3	219	227	VGYVSGWGR					Mascot
1044.5581	1044.5518	-0.0063	-6	203	211	VMPICLPSK			Carbamidomethyl (C)[5]		Mascot
1060.553	1060.5509	-0.0021	-2	203	211	VMPICLPSK			Carbamidomethyl (C)[5], Oxidation (M)[2]		Mascot
1146.5426	1146.5447	0.0021	2	253	262	HYEGSTVPEK					Mascot
1203.6368	1203.6368	0	0	333	342	VTSIQDWVQK					Mascot
1274.6376	1274.64	0.0024	2	253	263	HYEGSTVPEKK					Mascot
1290.7303	1290.7198	-0.0105	-8	157	168	DIAPTLTLYVGK					Mascot
1345.6456	1345.6458	0.0002	0	321	332	SCAVAEYGVYVK			Carbamidomethyl (C)[2]		Mascot

	1707.8193	1707.8247	0.0054	3	239	252	YVMLPVADQDQCIR			Carbamidomethyl (C)[12]				Mascot
	1723.8142	1723.8153	0.0011	1	239	252	YVMLPVADQDQCIR			Carbamidomethyl (C)[12], Oxidation (M)[3]				Mascot
	2188.0525	2188.0503	-0.0022	-1	267	286	SPVGVQPILNEHTFCAG MSK			Carbamidomethyl (C)[15], Oxidation (M)[18]				Mascot
2	HUMHPAB NID: - Homo sapiens							AAA52687		15	140	100	64 Mascot	100

Protein Group

haptoglobin precursor, allele 2 [validated] - human HPHU2

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
760.3987	760.3983	-0.0004	-1	292	297	FTDHLK					Mascot
809.3787	809.384	0.0053	7	271	277	DYAEVGR					Mascot
856.4675	856.5224	0.0549	64	113	118	NYYKLR					Mascot
858.493	858.4936	0.0006	1	229	235	QLVEIEK					Mascot
920.4624	920.4621	-0.0003	0	171	178	GSFPWQAK					Mascot
923.5308	923.5319	0.0011	1	162	170	ILGGHLDAK					Mascot
923.5308	923.5319	0.0011	1	162	170	ILGGHLDAK	64	100			Mascot
980.4948	980.4982	0.0034	3	278	286	VGYVSGWGR					Mascot
1044.5581	1044.5518	-0.0063	-6	262	270	VMPICLPSK			Carbamidomethyl (C)[5]		Mascot
1060.553	1060.5509	-0.0021	-2	262	270	VMPICLPSK			Carbamidomethyl (C)[5], Oxidation (M)[2]		Mascot
1146.5426	1146.5447	0.0021	2	312	321	HYEGSTVPEK					Mascot
1203.6368	1203.6368	0	0	392	401	VTSIQDWVQK					Mascot
1274.6376	1274.64	0.0024	2	312	322	HYEGSTVPEKK					Mascot
1290.7303	1290.7198	-0.0105	-8	216	227	DIAPTLTLYVGK					Mascot
1345.6456	1345.6458	0.0002	0	380	391	SCAVAEYGVYVK			Carbamidomethyl (C)[2]		Mascot
1707.8193	1707.8247	0.0054	3	298	311	YVMLPVADQDQCIR			Carbamidomethyl (C)[12]		Mascot
1723.8142	1723.8153	0.0011	1	298	311	YVMLPVADQDQCIR			Carbamidomethyl (C)[12], Oxidation (M)[3]		Mascot
2188.0525	2188.0503	-0.0022	-1	326	345	SPVGVQPILNEHTFCAG MSK			Carbamidomethyl (C)[15], Oxidation (M)[18]		Mascot

3	HSHP201 NID: - Homo sapiens	CAA25248		13	127	100	64 Mascot	100			

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank Result Type
760.3987	760.3983	-0.0004	-1	290	295	FTDHLK				Mascot
809.3787	809.384	0.0053	7	269	275	DYAEVGR				Mascot
856.4675	856.5224	0.0549	64	111	116	NYYKLR				Mascot
858.493	858.4936	0.0006	1	227	233	QLVEIEK				Mascot
920.4624	920.4621	-0.0003	0	169	176	GSFPWQAK				Mascot
923.5308	923.5319	0.0011	1	160	168	ILGGHLDAK				Mascot
923.5308	923.5319	0.0011	1	160	168	ILGGHLDAK	64	100		Mascot
980.4948	980.4982	0.0034	3	276	284	VGYVSGWGR				Mascot
1044.5581	1044.5518	-0.0063	-6	260	268	VMPICLPSK			Carbamidomethyl (C)[5]	Mascot
1060.553	1060.5509	-0.0021	-2	260	268	VMPICLPSK			Carbamidomethyl (C)[5], Oxidation (M)[2]	Mascot
1146.5426	1146.5447	0.0021	2	310	319	HYEGSTVPEK				Mascot
1274.6376	1274.64	0.0024	2	310	320	HYEGSTVPEKK				Mascot
1290.7303	1290.7198	-0.0105	-8	214	225	DIAPTLTLYVGK				Mascot
1707.8193	1707.8247	0.0054	3	296	309	YVMLPVADQDQCIR			Carbamidomethyl (C)[12]	Mascot
1723.8142	1723.8153	0.0011	1	296	309	YVMLPVADQDQCIR			Carbamidomethyl (C)[12], Oxidation (M)[3]	Mascot
2188.0525	2188.0503	-0.0022	-1	324	343	SPVGVQPILNEHTFCAG MSK			Carbamidomethyl (C)[15], Oxidation (M)[18]	Mascot

Gel Idx/Pos	39/B12	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	2

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	Similar to ribosomal protein L4.- Homo sapiens (Human).		Q9BSV5	18	111	100		Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3401	572.3467	0.0066	12	318	322	KPAEK					Mascot
576.3034	576.3574	0.054	94	102	105	MRNR					Mascot
579.3031	579.3358	0.0327	56	240	243	TMRR			Oxidation (M)[2]		Mascot
581.363	581.344	-0.019	-33	215	218	IHRR					Mascot
592.2983	592.3547	0.0564	95	102	105	MRNR			Oxidation (M)[1]		Mascot
596.3627	596.3614	-0.0013	-2	214	217	KIHR			Carbamyl (N-term)[0]		Mascot
606.314	606.3725	0.0585	96	240	243	TMRR			Carbamyl (N-term)[0]		Mascot
615.3685	615.3904	0.0219	36	108	111	IQRR			Carbamyl (N-term)[0]		Mascot
616.3776	616.3936	0.016	26	244	248	NTILR					Mascot
618.347	618.394	0.047	76	13	16	TWRR					Mascot
630.3933	630.4	0.0067	11	255	259	LRVDK					Mascot
632.3726	632.4002	0.0276	44	306	311	KAAATK			Carbamyl (N-term)[0]		Mascot
642.3933	642.3929	-0.0004	-1	222	226	KNPLK			Carbamyl (N-term)[0]		Mascot
694.3592	694.4009	0.0417	60	7	12	MFAPTK					Mascot
785.5131	785.4542	-0.0589	-75	72	78	EAVLLLK					Mascot
854.5206	854.5112	-0.0094	-11	223	229	NPLKNLR					Mascot
980.4981	980.494	-0.0041	-4	4	12	GGRMFAPTK			Oxidation (M)[4]		Mascot
1030.568	1030.5537	-0.0143	-14	80	87	LKAWNDIK			Carbamyl (N-term)[0]		Mascot

	1057.6616	1057.5664	-0.0952	-90	70	78	TKEAVLLLK			Carbamyl (N-term)[0]			Mascot
	1146.5361	1146.5441	0.008	7	181	189	SNYNLPMHK			Carbamyl (N-term)[0]			Mascot
2	ribosomal protein L4 - human							T09551	18	95	99.997	Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3401	572.3467	0.0066	12	411	415	KPAEK					Mascot
576.3034	576.3574	0.054	94	196	199	MRNR					Mascot
579.3031	579.3358	0.0327	56	333	336	TMRR			Oxidation (M)[2]		Mascot
581.363	581.344	-0.019	-33	308	311	IHRR					Mascot
592.2983	592.3547	0.0564	95	196	199	MRNR			Oxidation (M)[1]		Mascot
596.3627	596.3614	-0.0013	-2	307	310	KIHR			Carbamyl (N-term)[0]		Mascot
606.314	606.3725	0.0585	96	333	336	TMRR			Carbamyl (N-term)[0]		Mascot
615.3685	615.3904	0.0219	36	201	204	IQRR			Carbamyl (N-term)[0]		Mascot
616.3776	616.3936	0.016	26	337	341	NTILR					Mascot
618.347	618.394	0.047	76	107	110	TWRR					Mascot
630.3933	630.4	0.0067	11	348	352	LRVDK					Mascot
632.3726	632.4002	0.0276	44	399	404	KAAATK			Carbamyl (N-term)[0]		Mascot
642.3933	642.3929	-0.0004	-1	315	319	KNPLK			Carbamyl (N-term)[0]		Mascot
694.3592	694.4009	0.0417	60	101	106	MFAPTK					Mascot
785.5131	785.4542	-0.0589	-75	166	172	EAVLLLK					Mascot
854.5206	854.5112	-0.0094	-11	316	322	NPLKNLR					Mascot
980.4981	980.494	-0.0041	-4	98	106	GGRMFAPTK			Oxidation (M)[4]		Mascot
1030.568	1030.5537	-0.0143	-14	174	181	LKAWNDIK			Carbamyl (N-term)[0]		Mascot
1057.6616	1057.5664	-0.0952	-90	164	172	TKEAVLLLK			Carbamyl (N-term)[0]		Mascot
1146.5361	1146.5441	0.008	7	274	282	SNYNLPMHK			Carbamyl (N-term)[0]		Mascot

3	60S ribosomal protein L4 (L1).- Homo sapiens (Human).							RL4_HUMAN	18	95	99.997	Mascot	

Protein Group

BC001365 NID: - Homo sapiens AAH01365

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
572.3401	572.3467	0.0066	12	412	416	KPAEK					Mascot
576.3034	576.3574	0.054	94	196	199	MRNR					Mascot
579.3031	579.3358	0.0327	56	334	337	TMRR			Oxidation (M)[2]		Mascot
581.363	581.344	-0.019	-33	309	312	IHRR					Mascot
592.2983	592.3547	0.0564	95	196	199	MRNR			Oxidation (M)[1]		Mascot
596.3627	596.3614	-0.0013	-2	308	311	KIHR			Carbamyl (N-term)[0]		Mascot
606.314	606.3725	0.0585	96	334	337	TMRR			Carbamyl (N-term)[0]		Mascot
615.3685	615.3904	0.0219	36	202	205	IQRR			Carbamyl (N-term)[0]		Mascot
616.3776	616.3936	0.016	26	338	342	NTILR					Mascot
618.347	618.394	0.047	76	107	110	TWRR					Mascot
630.3933	630.4	0.0067	11	349	353	LRVDK					Mascot
632.3726	632.4002	0.0276	44	400	405	KAAATK			Carbamyl (N-term)[0]		Mascot
642.3933	642.3929	-0.0004	-1	316	320	KNPLK			Carbamyl (N-term)[0]		Mascot
694.3592	694.4009	0.0417	60	101	106	MFAPTK					Mascot
785.5131	785.4542	-0.0589	-75	166	172	EAVLLLK					Mascot
854.5206	854.5112	-0.0094	-11	317	323	NPLKNLR					Mascot
980.4981	980.494	-0.0041	-4	98	106	GGRMFAPTK			Oxidation (M)[4]		Mascot
1030.568	1030.5537	-0.0143	-14	174	181	LKAWNDIK			Carbamyl (N-term)[0]		Mascot
1057.6616	1057.5664	-0.0952	-90	164	172	TKEAVLLLK			Carbamyl (N-term)[0]		Mascot
1146.5361	1146.5441	0.008	7	275	283	SNYNLPMHK			Carbamyl (N-term)[0]		Mascot

Gel Idx/Pos	40/B13	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	2

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	Hypothetical protein (Fragment).-	Homo sapiens (Human).	Q8NDT6	16	63	95.479		Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Start Seq.	End Seq.	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
582.3106	582.2962	-0.0144	-25	357	360	NHLR			Carbamyl (N-term)[0]		Mascot
584.3151	584.3564	0.0413	71	211	215	QSPPR					Mascot
597.3215	597.3718	0.0503	84	239	242	HERR					Mascot
615.3572	615.3688	0.0116	19	661	665	QSPKR					Mascot
620.2998	620.3584	0.0586	94	78	82	TNQSK			Carbamyl (N-term)[0]		Mascot
689.3325	689.3771	0.0446	65	498	502	DERAR			Carbamyl (N-term)[0]		Mascot
807.3525	807.3972	0.0447	55	382	387	NDRMGR			Carbamyl (N-term)[0], Oxidation (M)[4]		Mascot
840.4938	840.4917	-0.0021	-2	187	193	TLTPPLR			Carbamyl (N-term)[0]		Mascot
856.4886	856.524	0.0354	41	31	38	LSPSPSLR					Mascot
927.4166	927.5006	0.084	91	309	315	DYSRDTK			Carbamyl (N-term)[0]		Mascot
935.4581	935.5513	0.0932	100	179	186	TPSPSYQR					Mascot
1027.5895	1027.5581	-0.0314	-31	31	39	LSPSPSLRK			Carbamyl (N-term)[0]		Mascot
1033.5021	1033.5453	0.0432	42	374	381	NESRSEIR			Carbamyl (N-term)[0]		Mascot
1034.4609	1034.5292	0.0683	66	370	377	NESRNESR			Carbamyl (N-term)[0]		Mascot
1106.5437	1106.5392	-0.0045	-4	361	369	EESSRTEIR					Mascot
1127.5552	1127.5559	0.0007	1	357	365	NHLREESSR					Mascot

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
2	Hypothetical protein (Fragment).-	Homo sapiens	Q8TAK3	9	61	93.157		Mascot	

(Human).

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
672.3787	672.4017	0.023	34	76	80	VNQLR			Carbamyl (N-term)[0]		Mascot
689.3576	689.3771	0.0195	28	71	75	QEREK					Mascot
785.474	785.4529	-0.0211	-27	163	168	INRAIR			Carbamyl (N-term)[0]		Mascot
1033.4553	1033.5453	0.09	87	58	66	NMSGHVSMK			Carbamyl (N-term)[0]		Mascot
1035.5503	1035.5166	-0.0337	-33	49	57	TLGSLMNIK			Carbamyl (N-term)[0], Oxidation (M)[6]		Mascot
1057.4983	1057.5638	0.0655	62	20	29	FGSAMEKSGK			Oxidation (M)[5]		Mascot
1127.5327	1127.5559	0.0232	21	172	180	ESVEEHELR					Mascot
1179.6005	1179.5962	-0.0043	-4	102	110	EELLRGYEK			Carbamyl (N-term)[0]		Mascot
1398.6934	1398.6097	-0.0837	-60	120	131	VSLEMYQSQVAK			Oxidation (M)[5]		Mascot

3 ALS2CR11 protein (Fragment).- Homo sapiens (Human). Q96Q36 11 56 77.34 Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
570.3358	570.3414	0.0056	10	157	161	IPNAR					Mascot
590.3079	590.3485	0.0406	69	414	417	MRPK			Carbamyl (N-term)[0], Oxidation (M)[1]		Mascot
613.3416	613.362	0.0204	33	157	161	IPNAR			Carbamyl (N-term)[0]		Mascot
620.3799	620.3584	-0.0215	-35	124	128	TLKMK					Mascot
856.4635	856.524	0.0605	71	282	288	DRVAQPK			Carbamyl (N-term)[0]		Mascot
1035.5292	1035.5166	-0.0126	-12	119	126	AWCEKTLK			Carbamidomethyl (C)[3]		Mascot
1106.5994	1106.5392	-0.0602	-54	213	221	HLVTFAQYK					Mascot
1193.6677	1193.6292	-0.0385	-32	19	27	KYFPLQNLK			Carbamyl (N-term)[0]		Mascot
1194.5247	1194.6145	0.0898	75	358	367	SSDDIHNHAR			Carbamyl (N-term)[0]		Mascot

1365.6216	1365.6464	0.0248	18	162	171	MPREDEYLNR	Carbamyl (N-term)[0]	Mascot
1638.7759	1638.9174	0.1415	86	100	113	TEIQDPYSWGGKSK	Carbamyl (N-term)[0]	Mascot
2225.1965	2225.1311	-0.0654	-29	252	269	VVEDEKNLKPHLFPELFK	Carbamyl (N-term)[0]	Mascot

Gel Idx/Pos	41/B14	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	2

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	APOA1 PROTEIN (FRAGMENT).- Homo sapiens (Human).		CAA00975	17	123	100		Mascot	

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
573.3606	573.4141	0.0535	93	178	182	LEALK					Mascot
613.3668	613.3699	0.0031	5	119	123	VEPLR					Mascot
615.3824	615.3845	0.0021	3	41	45	QLNLK					Mascot
781.4315	781.4335	0.002	3	154	160	AHVDALR					Mascot
831.4359	831.4446	0.0087	10	189	195	LAEYHAK					Mascot
869.5203	869.5251	0.0048	6	117	123	QKVEPLR					Mascot
873.4424	873.468	0.0256	29	124	131	AELQEGAR					Mascot
896.4836	896.4844	0.0008	1	134	140	LHELQEK					Mascot
1012.5785	1012.5853	0.0068	7	207	215	AKPALEDLR					Mascot
1031.519	1031.5405	0.0215	21	141	149	LSPLGEEMR					Mascot
1215.6216	1215.62	-0.0016	-1	196	206	ATEHLSTLSEK					Mascot
1226.5437	1226.548	0.0043	4	1	10	DEPPQSPWDR					Mascot
1252.6208	1252.6184	-0.0024	-2	97	106	VQPYLDDFQK					Mascot
1258.6273	1258.5408	-0.0865	-69	196	206	ATEHLSTLSEK			Carbamyl (N-term)[0]		Mascot
1299.5674	1299.5695	0.0021	2	108	116	WQEEMELYR			Oxidation (M)[5]		Mascot
1301.6484	1301.6517	0.0033	3	161	171	THLAPYSDELR					Mascot
1318.642	1318.6448	0.0028	2	141	151	LSPLGEEMRDR			Oxidation (M)[8]		Mascot
1400.6692	1400.6653	-0.0039	-3	28	40	DYVSQFEGSALGK					Mascot

2	HUMAPOAIC NID: - Homo sapiens	AAA51747	16	110	100	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
573.3606	573.4141	0.0535	93	184	188	LEALK					Mascot
613.3668	613.3699	0.0031	5	125	129	VEPLR					Mascot
615.3824	615.3845	0.0021	3	47	51	QLNLK					Mascot
781.4315	781.4335	0.002	3	160	166	AHVDALR					Mascot
831.4359	831.4446	0.0087	10	195	201	LAEYHAK					Mascot
869.5203	869.5251	0.0048	6	123	129	QKVEPLR					Mascot
873.4424	873.468	0.0256	29	130	137	AELQEGAR					Mascot
896.4836	896.4844	0.0008	1	140	146	LHELQEK					Mascot
1012.5785	1012.5853	0.0068	7	213	221	AKPALEDLR					Mascot
1031.519	1031.5405	0.0215	21	147	155	LSPLGEEMR					Mascot
1215.6216	1215.62	-0.0016	-1	202	212	ATEHLSTLSEK					Mascot
1252.6208	1252.6184	-0.0024	-2	103	112	VQPYLDDFQK					Mascot
1258.6273	1258.5408	-0.0865	-69	202	212	ATEHLSTLSEK			Carbamyl (N-term)[0]		Mascot
1299.5674	1299.5695	0.0021	2	114	122	WQEEMELYR			Oxidation (M)[5]		Mascot
1301.6484	1301.6517	0.0033	3	167	177	THLAPYSDELR					Mascot
1318.642	1318.6448	0.0028	2	147	157	LSPLGEEMRDR			Oxidation (M)[8]		Mascot
1400.6692	1400.6653	-0.0039	-3	34	46	DYVSQFEGSALGK					Mascot

3	apolipoprotein A-I precursor [validated] - human	LPHUA1	16	106	100	Mascot

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type

573.3606	573.4141	0.0535	93	202	206	LEALK		Mascot
613.3668	613.3699	0.0031	5	143	147	VEPLR		Mascot
615.3824	615.3845	0.0021	3	65	69	QLNLK		Mascot
781.4315	781.4335	0.002	3	178	184	AHVDALR		Mascot
831.4359	831.4446	0.0087	10	213	219	LAEYHAK		Mascot
869.5203	869.5251	0.0048	6	141	147	QKVEPLR		Mascot
873.4424	873.468	0.0256	29	148	155	AELQEGAR		Mascot
896.4836	896.4844	0.0008	1	158	164	LHELQEK		Mascot
1012.5785	1012.5853	0.0068	7	231	239	AKPALEDLR		Mascot
1031.519	1031.5405	0.0215	21	165	173	LSPLGEEMR		Mascot
1215.6216	1215.62	-0.0016	-1	220	230	ATEHLSTLSEK		Mascot
1252.6208	1252.6184	-0.0024	-2	121	130	VQPYLDDFQK		Mascot
1258.6273	1258.5408	-0.0865	-69	220	230	ATEHLSTLSEK	Carbamyl (N-term)[0]	Mascot
1299.5674	1299.5695	0.0021	2	132	140	WQEEMELYR	Oxidation (M)[5]	Mascot
1301.6484	1301.6517	0.0033	3	185	195	THLAPYSDELR		Mascot
1318.642	1318.6448	0.0028	2	165	175	LSPLGEEMRDR	Oxidation (M)[8]	Mascot
1400.6692	1400.6653	-0.0039	-3	52	64	DYVSQFEGSALGK		Mascot

Gel Idx/Pos	42/B15	Instr./Gel Origin	maldi222/David Haarburger	Process Status	Analysis Succeeded
Plate [#] Name	[1] David Haarburger	Instrument Sample Name		Spectra	4

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
1	hemoglobin beta subunits are s-nitrosylated. the heme groups are nitrosylated., chain B - human (fr		1BUWB	7	112	100	70	Mascot	100

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
583.331	583.3383	0.0073	13	62	66	AHGKK			Carbamyl (N-term)[0]		Mascot
952.5098	952.5107	0.0009	1	1	8	VHLTPEEK					Mascot
1126.564	1126.5649	0.0009	1	96	104	LHVDPENFR					Mascot
1149.6738	1149.668	-0.0058	-5	133	144	VVAGVANALAHK					Mascot
1274.7256	1274.7211	-0.0045	-4	31	40	LLVVYPWTQR					Mascot
1314.6648	1314.6659	0.0011	1	18	30	VNVDEVGGEALGR	70	100			Mascot
1314.6648	1314.6659	0.0011	1	18	30	VNVDEVGGEALGR			Carbamyl (N-term)[0]		Mascot
1378.7001	1378.6954	-0.0047	-3	121	132	EFTPPVQAAYQK					Mascot

Rank	Protein Name	Species	Accession No.	Pep. Count	Protein Score	Protein Score C. I. %	Total Ion Score	Result Type	Total Ion Confirmed C. I. %
2	hemoglobin beta chain - pygmy chimpanzee		HBCZP	7	111	100	70	Mascot	100

Protein Group

AF083883 NID: - Homo sapiens	AAL68978
AF117710 NID: - Homo sapiens	AAD19696
AF181989 NID: - Homo sapiens	AAF00489
deshis deoxyhemoglobin a mutant HIS 146 BETA REMOVED, chain B - human	1AXFB
hemoglobin beta chain [validated] - human	HBHU

hemoglobin mutant CHAIN B, D, C112G alpha-oxy, beta-deoxy, t state, chain B - human 1GBVB

hemoglobin mutant CHAIN B, D, C93A, C112G deoxy, chain B - human 1GBUB

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
583.331	583.3383	0.0073	13	62	66	AHGKK			Carbamyl (N-term)[0]		Mascot
952.5098	952.5107	0.0009	1	1	8	VHLTPEEK					Mascot
1126.564	1126.5649	0.0009	1	96	104	LHVDPENFR					Mascot
1149.6738	1149.668	-0.0058	-5	133	144	VVAGVANALAHK					Mascot
1274.7256	1274.7211	-0.0045	-4	31	40	LLVVYPWTQR					Mascot
1314.6648	1314.6659	0.0011	1	18	30	VNVDEVGGEALGR	70	100			Mascot
1314.6648	1314.6659	0.0011	1	18	30	VNVDEVGGEALGR			Carbamyl (N-term)[1,5]		Mascot
1378.7001	1378.6954	-0.0047	-3	121	132	EFTPPVQAAYQK					Mascot

3 Hemoglobin (deoxy) mutant with val b 1 replaced by met, his B 2 deleted, val d 1 replaced by met, a 2HHEB 7 111 100 70 Mascot 100

Peptide Information

Calc. Mass	Obsrv. Mass	± da	± ppm	Star Seq	End Seq	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
583.331	583.3383	0.0073	13	61	65	AHGKK			Carbamyl (N-term)[0]		Mascot
759.3882	759.4499	0.0617	81	2	7	LTPEEK			Carbamyl (N-term)[0]		Mascot
1126.564	1126.5649	0.0009	1	95	103	LHVDPENFR					Mascot
1149.6738	1149.668	-0.0058	-5	132	143	VVAGVANALAHK					Mascot
1274.7256	1274.7211	-0.0045	-4	30	39	LLVVYPWTQR					Mascot
1314.6648	1314.6659	0.0011	1	17	29	VNVDEVGGEALGR	70	100			Mascot
1314.6648	1314.6659	0.0011	1	17	29	VNVDEVGGEALGR					Mascot
1378.7001	1378.6954	-0.0047	-3	120	131	EFTPPVQAAYQK					Mascot