PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/53, 33/68

A1

(11) International Publication Number: **WO 98/39652**

(43) International Publication Date: 11 September 1998 (11.09.98)

(21) International Application Number: PCT/SE98/00351

(22) International Filing Date: 27 February 1998 (27.02.98)

(30) Priority Data:
9700745-4 3 March 1997 (03.03.97) SE

(71) Applicant *(for all designated States except US)*: PHARMACIA & UPJOHN DIAGNOSTICS AB [SE/SE]; S-751 82 Uppsala (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SILLANAUKEE, Pekka [FI/SE]; Tornugglevägen 21, S-756 53 Uppsala (SE). HURME, Liisa [FI/SE]; Marielundsgatan 3E, S-753 25 Uppsala (SE). WORRALL, Simon [AU/AU]; 28 McPherson Road, Sinnamon Park, QLD 4073 (AU).

(74) Agents: BERGANDER, Håkan et al.; Pharmacia & Upjohn AB, Patent Dept., S-751 82 Uppsala (SE).

(81) Designated States: AU, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: IMMUNOASSAY AND KIT FOR IgA ANTIBODIES SPECIFIC FOR ACETALDEHYDE ADDUCTS

(57) Abstract

An immunoassay method for detecting acetaldehyde protein adduct specific IgA antibody in body fluid samples employing an acetaldehyde adduct as the antigen, characterized in that the adduct is between acetaldehyde and a fragment of a native protein. A diagnostic method for detecting heavy drinkers and alcoholics utilizing the immunoassay method. A test kit useful for the immunoassay and the diagnostic method comprising anti-IgA antibody and an acetaldehyde adduct with a fragment of a native polypeptide.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

IMMUNOASSAY AND KIT FOR IgA ANTIBODIES SPECIFIC FOR ACETALDEHYDE ADDUCTS

The present invention concerns an improved immunoassay method for the measurement of IgA antibodies directed against acetaldehyde adducts of proteins. This type of assays is useful for the diagnosis of high alcohol intake including both non-alcoholic heavy drinkers and alcoholics.

Recently, the presence of antibodies reactive with acetaldehyde modified protein epitopes in the plasma of alcoholics has been linked to alcohol abuse. However, these antibodies have also been observed in patients with non-alcoholic liver disease and are as such considered unspecific for high alcohol intake (Niemela et al., Hepatology 7 (1987) 1210-1214; Hoerner et al., Hepatology 8 (1988) 569-574; Worrall et al., Alcohol Alcoholism 25 (1990) 509-511). IgA class antibodies against acetaldehyde modified proteins have been shown to be elevated in alcoholics (Worrall et al., Eur. L. Clin. Invest. 21 (1991) 90-95) and heavy drinkers (Worrall et al., Alcoholism: Clin. Exp. Res. 20(1996) 836-840) as compared to social drinkers, and in contrast to total Ig reactivity, IgA reactivity was not elevated in non-alcohol related liver disease.

The methods used for determining IgA antibody titres against protein acetaldehyde adducts have utilized immunoassays involving formation and detection/determination of the complex:

anti-IgA -- sample IgA -- adduct

Adduct stands for an <u>in vitro</u> prepared acetaldehyde adduct of a protein and it functions as an antigen in the assay. The protein used for synthesis of the adduct has been bovine serum albumin or bovine hemoglobin. Sample IgA is self-explanatory. Anti-IgA stands for added antibodies specific for IgA. It has been found that the correlation to high alcohol intake will be improved in case one uses adduct specific reactivity (ASR) which is obtained by subtracting the IgA titre obtained with the unmodified antigen from the IgA titre obtained with the

corresponding adduct (Worrall et al., Alcoholism: Clin. Exp. Res. 20(1996) 836-840).

Acetaldehyde, the primary metabolite of ethanol, has been shown to accumulate in the blood and liver following chronic ethanol intake (Nuutinen et al., Alcoholism: Clin. Exp. Res. 7 (1983) 163-168 and Nuutinen et al., Eur. J. Clin. Invest. 14 (1984) 306). Multiple forms of acetaldehyde adducts of various stability are formed in vitro with proteins (Donohue et al., (Arch. Biochem. Biophys 220 (1983) 239-246), lipids, nucleic acids and carbohydrates (Nicholls et al., Int. J. Biochem. 24 (1992) 1899-1906) are formed in vitro. Hemoglobin adducts formed in vitro have been detected by Sillanaukee et al (Alcohol. Clin. Exp. Res. 14 (1990) 842-846), Hazelett et al (Alcohol. Clin. Exp. Res. 17 (1992) 1107-1111) and Itälä et al (Anal. Biochem. 224 (1995) 323-329), and in vivo by Sillanaukee et al (Alcohol Alcoholism. 26 (1991) 5-6); Sillanaukee et al (Alcohol. 8 (1991) 377-381) and Sillanaukee et al J. Lab. Clin. Med. 120 (1992) 42-47).

Serum/plasma levels of acetaldehyde protein adducts have been measured by the use of ELISA methods employing polyclonal antibodies (Lin et al., Alcohol. Clin. Exp. Res. 3 (1993) 669-674; Sillanaukee et al J. Lab. Clin. Med. 120 (1992) 42-47; Niemelä and Israel, Lab. Invest. 67 (19992) 246-252; Niemelä et al., Alcohol. Clin. Exp. Res. 14 (1990) 838-841; and Lin et al., Alcohol. Clin. Exp. Res. 14 (1990) 438-443) produced against acetaldehyde adducts of hemoglobin or against synthetic peptides designed to mimic the sequences of the hemoglobin β-chain, which are theoretically reactive with acetaldehyde (Lin et al., Alcohol. Clin. Exp. Res. 3 (1993) 669-674). Monoclonal antibodies against synthetic peptide adducts have been described (Klassen et al., Alcohol. Clin. Exp. Res. 18 (1994) 164-171; Thiele et al., Biochem. Pharmacol. 48 (1994) 183-189; and Lin et al., Alcohol. Clin. Exp. Res. 19 (1995) 314-319). The use of monoclonal antibodies for measuring clinical relevant serum/plasma levels of acetaldehyde adducts has so far not been reported.

Acetaldehyde may react with primary and secondary amino groups in proteins, i.e. lysine and the N-terminal amino group of proteins. The initial product is a Schiff base that may be stabilised by further reaction to an ethylated amine, or, in N-terminal amino groups, also to a 2-methyl-imidazolidine-4-one. Adduct formation with amino terminal peptides derived from the hemoglobin β-chain (VHLTPEK and VHLTPEC) under various conditions have been studied (Lin et al., Alcohol. Clin. Exp. Res. 19 (1995) 314-319), and Sillanaukee et al., Eur. J. Biochem. 240 (1996) 30-36).

It remains to be clarified which protein part sequences are important for forming acetaldehyde adducts in vivo, and which adduct structures are immunogenic in the sense that they create an elevated IgA reactivity due to high alcohol intake.

Objectives of the invention

A first objective of the invention is to provide synthetic well-defined and simplified acetaldehyde adducts that can be used for measuring human IgA antibodies specific for acetaldehyde protein adducts produced in vivo as a consequence of high alcohol intake in alcoholics and in heavy drinkers.

A second objective is to minimize sample IgA reactivity to the unmodified antigen used in the assay.

A third objective is to provide improved diagnostic methods for high alcohol intake.

The invention

We have now realized that these objectives can be achieved in case the adduct between acetaldehyde and a native protein is replaced with an adduct between acetaldehyde and a fragment of a native protein.

Accordingly the main aspect of the invention is an immunoassay method as defined below for determining body fluid IgA antibodies specific for acetaldehyde adducts. The characteristic feature is that an adduct between acetaldehyde and a fragment of a native protein is used as the antigen.

The most useful proteins are albumin and hemoglobin, i.e. the proteins that are most abundantly present in the body fluid contemplated. Fragments of human proteins are preferred.

By fragment is meant a part sequence of a native protein/polypeptide. The fragment may be obtained either by cleavage of a longer peptide chain followed by isolation of distinct fragment(s) or by direct recombinant production or chemical peptide synthesis of the intended fragment. With the present knowledge the most preferred fragments derive from amino terminal ends of native proteins or lysine and/or tyrosine containing surface exposed parts of native proteins. Useful amino-terminal fragments may, for instance, be found in the β-chain of hemoglobin. See for instance San George and Haberman, J. Biol. Chem. 261 (1986) 6811-6821 and Stevens et al., J. Clin. Invest. 67 (1981) 361-369. The length of the fragments may vary from 5 amino acid residues (with at least one residue providing a free amino group also present in the native protein) and upwards. Typically the fragments to be used are below 50%, such as below 10%, of the entire native polypeptide chain. At the present stage it is preferred to use fragments comprising native sequences of 5-10 amino acid residues.

The fragments may be covalently linked to analytically detectable groups or bound to carriers as described below. Spacer groups may have been inserted between the fragment and a label or a carrier in order to retain the efficient antibody binding activity of the fragment. Example of spacers are oligopeptides, preferably hydrophilic, not deriving from the native protein.

Acetaldehyde adducts may be synthesized by reacting the fragment with acetaldehyde under the appropriate conditions, normally either reducing or non-reducing. Reducing conditions results in adducts that comprise ethyl amino groups while the non-reducing conditions result in Schiff bases and optionally also in 2-methyl-imidazolidine-4-one structures. For further details see the experimental part and previous works that have

involved adduct formation (Sillanaukee et al (Eur. J. Biochem. 240 (1996) 30-36); Sillanaukee et al (J. Lab. Clin. Med. 120 (1992) 42-47); Lin et al (Alcohol. Clin. Exp. Res. 17 (1993) 882-886); Thiele et al (Biochem. Pharmacol 48 (1994) 183-189); and Klassen et al (Alcohol. Clin. Exp. Res. 18 (1994) 164-171). Lin et al (Alcohol. Clin. Exp. Res. 19 (1995) 314-319).

Conventional antigen specific IgA immunoassays can be used. All of them encompass the formation of a ternary immune complex

anti-IgA --- IgA --- antigen/hapten

where IgA is the acetaldehyde specific antibody to be determined in the sample and antigen/hapten correspond to the adduct as defined above. Anti-IgA or the adduct may me used in an insoluble form or an insolubilizable form as known in the art. Insoluble forms include that the IgA or the adduct is stably linked to a carrier, such as solid phase, for instance a tube wall, a microtiter well, a monolithic or a particulate carrier etc. Monolithic and particulate carriers should preferentially be hydrophilic and/or porous. In order to enable measurement of the ternary complex either anti-IgA or the adduct may be labelled with an analytically detectable group, such as an isotope, an enzyme, an enzyme substrate, an enzyme cofactor, a fluorophor, a chromophor, a chemiluminescent group, a particle, biotin, hapten etc. Immunoassay variants utilizing labelled reactants in combination with insoluble or insolubilizable immune reactants and a separation of labelled reactant bound to the insoluble or insolubilizable immune reactants from labelled reactant not bound to the insoluble or insolubilizable immune reactants are often categorized as heterogeneous assays. Another type of immunoassays is called homogeneous and mostly comprises only soluble reactants without any need for separation. Nephelometry or biosensor techniques may be used for detecting the formation of the above-mentioned ternary complex with or without the use insoluble or insolubilizable or labelled reactants.

The conditions and order of addition of the various reactants are in principle the same as for the above-mentioned

conventional assays, i.e. the sample containing IgA antibodies of the sought specificity is incubated with anti-IgA antibody and an acetaldehyde adduct of a protein fragment so that the ternary complex is formed. The amount selected of each reagents, order of addition, incubation times, temperature, pH etc are selected so as to allow for the complex to form in an amount that is related to the amount of sample IgA specific for the adduct. In the invention this means that anti-IgA and adduct normally should be in excess. Typical orders of additions are: adduct, sample, anti-IgA; anti-IgA, sample, adduct; sample, anti-IgA, adduct; adduct, anti-IgA, sample; sample, adduct, anti-IgA; and anti-IgA, adduct, sample. In case either the anti-IgA or the adduct is insoluble or insolubilizable it becomes possible to separate the immune complex formed in one step from excess reagents before adding the subsequent reagents. Separation procedures may encompass washing steps in order to more effectively remove excess components used in a prior step.

Typical reactions times for each incubation step are often in the range of one or a few minutes up to several hours. Even over-night incubations may be used if required. The exact time depends on factors such as avidity/affinity of anti-IgA, pH, temperature, type of solid phase (if any). In case insoluble forms are used, for instance anti-IgA or the adduct is attached to a solid phase, very short incubation times may be accomplished in case the solid phase is in a porous form that is capable of absorbing the complete volume of the incubation mixture and have pore sizes optimized for diminishing diffusion times at the pore surfaces (see for instance US 4,708,932 (Pharmacia AB).

The temperature should be in the range of 0-40°C, with preference for 15-40°C. The pH is normally in the range of 3-11, with preference for 4-10. It is imperative that both the pH and the temperature are selected so that the conditions are non-denaturing for the reactants involved.

The body fluid sample may be derived from saliva, blood, serum or plasma, or any other body fluid that may contain adduct specific IgA antibodies

One aspect of the invention is a diagnostic method employing an immunoassay wherein an acetaldehyde adduct of a protein fragment as defined above is used. Elevated levels of IgA-titres (compared to the level for healthy normal non-alcoholic consumers) are taken as an indication of high alcohol intake.

One further aspect of the invention is a kit containing anti-IgA antibody and an acetaldehyde adduct of a fragment of a native protein as defined above. The anti-IgA antibody and the adduct may be labelled, insoluble or isolubilized as described above. They may be in soluble and/or predispensed form or in dry form, for instance lyophilized or spray dried, ready to be reconstituted in the appropriate buffer for the assay contemplated.

Best mode

The best mode of the invention at the filing date is represented in the experimental part.

EXPERIMENTAL PART

Example 1 A: Synthesis of the acetaldehyde of a protein fragment (the five first amino acids of the N-terminal end of human hemoglobin plus a carboxy terminating cysteine (VHLTPEC).

The peptide was synthesized as earlier described (Sillanaukee et al., Eur. J. Biochem. 249 (1996) 30-46). The peptide was dissolved to 0.5 mg/ml in PBS pH 7.4. To 10 ml of the solution was then added 5 ml of 2.5 mM acetaldehyde dissolved in PBS and 5 ml of 250 mM sodium cyanoborohydride ($NaCNBH_3$) in PBS. The mixture was then allowed to incubate at ambient temperature for 48 hours in a tightly sealed container. The peptide solution can be stored at -20°C to +4°C in 0.5 ml aliquots.

Example 1B: Synthesis of the adduct with bovine serum albumin. The adduct was prepared as described in Worrall et al., Alcoholism: Clin. Exp. Res. 20 (1996) 836-840.

Example 1C: Synthesis of the adduct with human hemoglobin The adduct was prepared as described for bovine serum albumin in Worrall et al., Eur. J. Clin. Invest. 21 (1991) 90-95.

Example 2: Assay of IgA reactive antibodies in serum from patients and controls.

Patients: Sera was collected from non-alcoholic women and men with a well documented drinking history attending a voluntary health screening and from alcoholics on admission to a detoxification centre in the city of Tampere, Finland. The sera had been previously analysed in ELISA using acetaldehyde modified BSA as the coating antigen (Worrall et al., Alcoholism: Clin. Exp. Res. 20 (1996) 836-840). Based on the ELISA results 10 samples with a high IgA adduct specific reactivity (ASR) were chosen as positive controls and 10 samples with low IgA adduct specific reactivity (ASR) were chosen as negative controls.

Results: Figure 1 represents the result of Example 2a (peptide adduct). Figure 2 represents the results of Example 2B (hemoglobin adduct, Figure 3 represents the results of Example 3B (albumin adduct).

Example 2A. Assay protocol - peptide adduct:

Preparation of microtiter wells: Aliquots of 100 µl of modified and unmodified peptide (50 µg/ml), respectively, in sodium bicarbonate buffer pH 9.6 were added to the wells of a microtiter plate and allowed to incubate at room temperature for 1 hour. The wells were then washed 4x with PBS-Tween followed by addition to each well of 200 µl of blocking solution (1 % BSA in PBS, pH 7.4) and incubation for one hour at room temperature, whereafter the wells were washed 4x with PBS-Tween.

Assay: 100 µl of patient serum diluted 1:20 (10 % chicken serum - PBS) were added to each well and incubated for 1 hour at room temperature, whereafter the wells were washed 4x with PBS-Tween. To each well was added 100 µl of β-galactosidase-Fab anti-IgA antibody conjugate (2.64 µg/ml) followed by incubation for 1 hour at 37°C and washing 4x with PBS-Tween. 100 µl of o-nitrophenyl-β-galactoside were then added to each well and allowed to incubate for 1 hour at 37°C. 100 µl stop solution was the added and the absorbance read (405 nm).

The results are shown in Figures 1a-c. Figure 1a shows the results with unmodified peptide, figure 1b the result with modified peptide (AA) and figure 1c the result if the absorbance for unmodified peptide is subtracted from the absorbance for modified peptide (adduct specific reactivity = ASR).

Example 2B. Assay protocol - hemoglobin adduct.

The protocol is the same as for the peptide adduct except that the peptide antigen is replaced with the corresponding adduct of hemoglobin (Example 1C). The results are presented in Figure 2a-c. Each of the figures 2a-c has correspondence in respective figure 1a-c.

Example 2C. Assay protocol - albumin adduct.

The protocol is the same as for the peptide adduct except that the peptide antigen is replaced with the corresponding adduct of bovine serum albumin (Example 1B). The results are presented in Figure 3a-c. Each of the figures 3a-c has correspondence in respective figure 1a-c.

Conclusions

The results indicates that adducts of protein fragments are superior to protein adducts. Firstly, the unspecific binding to carrier protein, which can be seen with the hemoglobin-acetaldehyde adduct, could be avoided by using a peptide adduct. Secondly, the peptide adduct showed better sensitivity

and specificity as compared to the BSA-adduct. Thirdly, the fact that alcoholics and controls did not show any significant difference in reactivity to unmodified peptide might enable the use of AA-values alone.

PATENT CLAIMS

1. An immunoassay method for detecting acetaldehyde protein adduct specific IgA antibody in body fluid samples employing an acetaldehyde adduct as the antigen, **characterized** in that the adduct is between acetaldehyde and a fragment of a native protein.

2. The method according to claim 1, **characterized** in that the native protein is hemoglobin.

3. The method according to anyone of claims 1-2, **characterized** in that the fragment is N-terminal in the native protein.

4. The method according to anyone of claims 1-3, **characterized** in that the immunoassay employs formation of the ternary complex

anti-IgA -- IgA -- adduct

where IgA stands for adduct specific IgA antibody in the sample and anti-IgA and adduct are added reagents.

5. The method according to claim 4, **characterized** in that the adduct is bound to a solid phase and that anti-IgA is labelled.

6. A method for diagnosing high alcohol intake, **characterized** in that the method of anyone of claims 1-5 is used and that an increased level in a sample from an individual, compared to healthy normal alcohol consumers, is taken as an indication of high alcohol intake.

7. Test kit containing anti-IgA antibody and an acetaldehyde adduct with a fragment of a native protein.

8. Test kit according to claim 7, **characterized** in that the fragment is amino terminal in the native protein.

9. Test kit according to any of claims 7-8, **characterized** in that the native protein is albumin or hemoglobin.

Figure 1

IgA reactivity to monoethylated peptide (m/z 826) of hemoglobin B-chain N-terminus measured by IgA ASR EIA

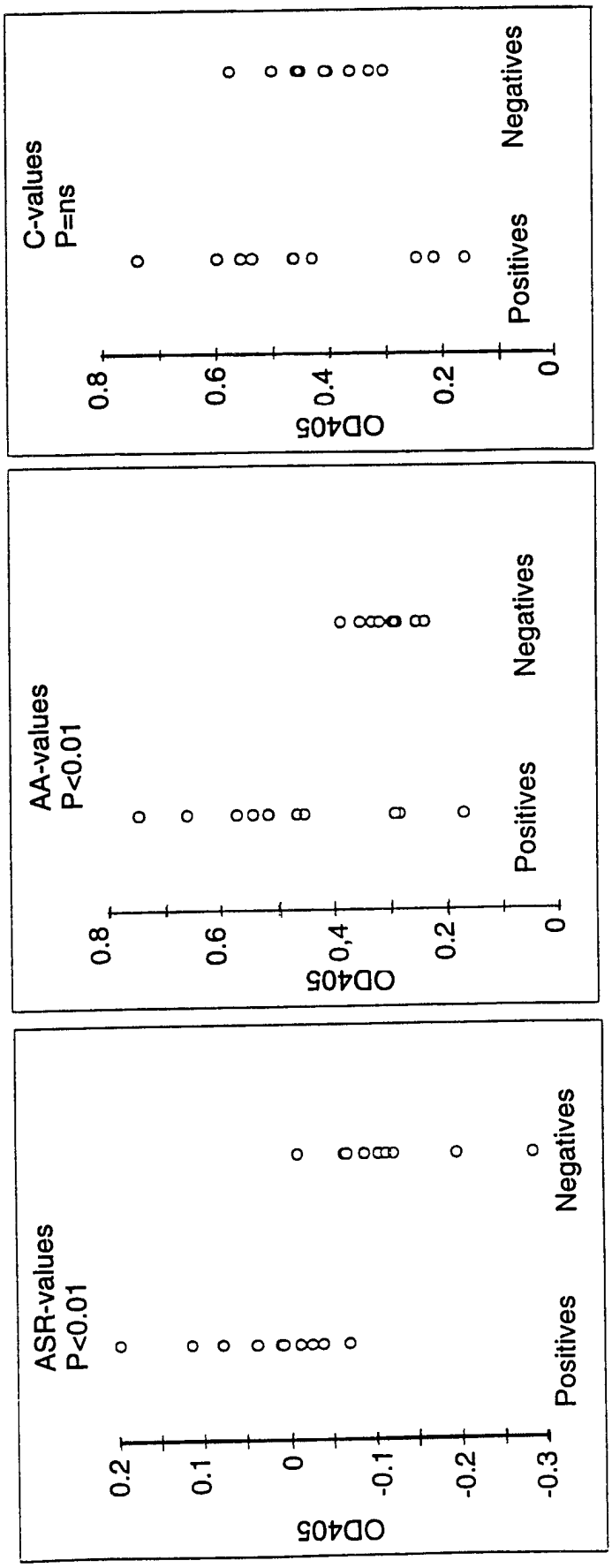


Figure 2

IgA reactivity to hemoglobin-antigen measured by IgA ASR EIA

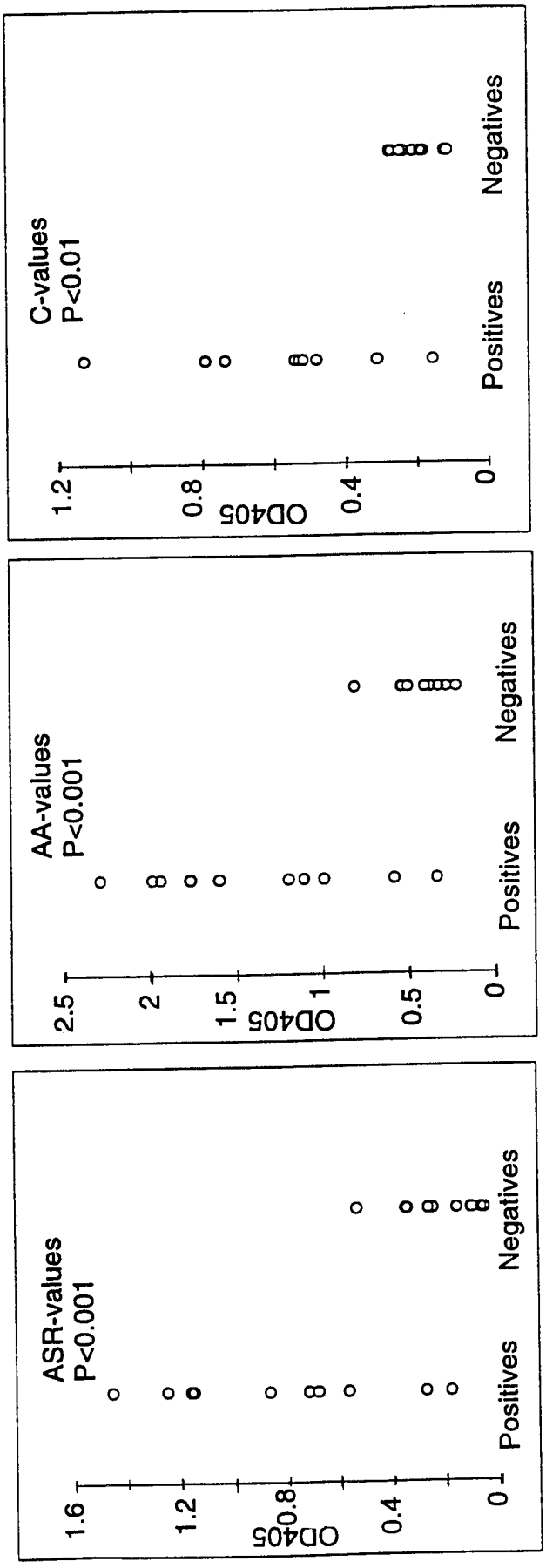
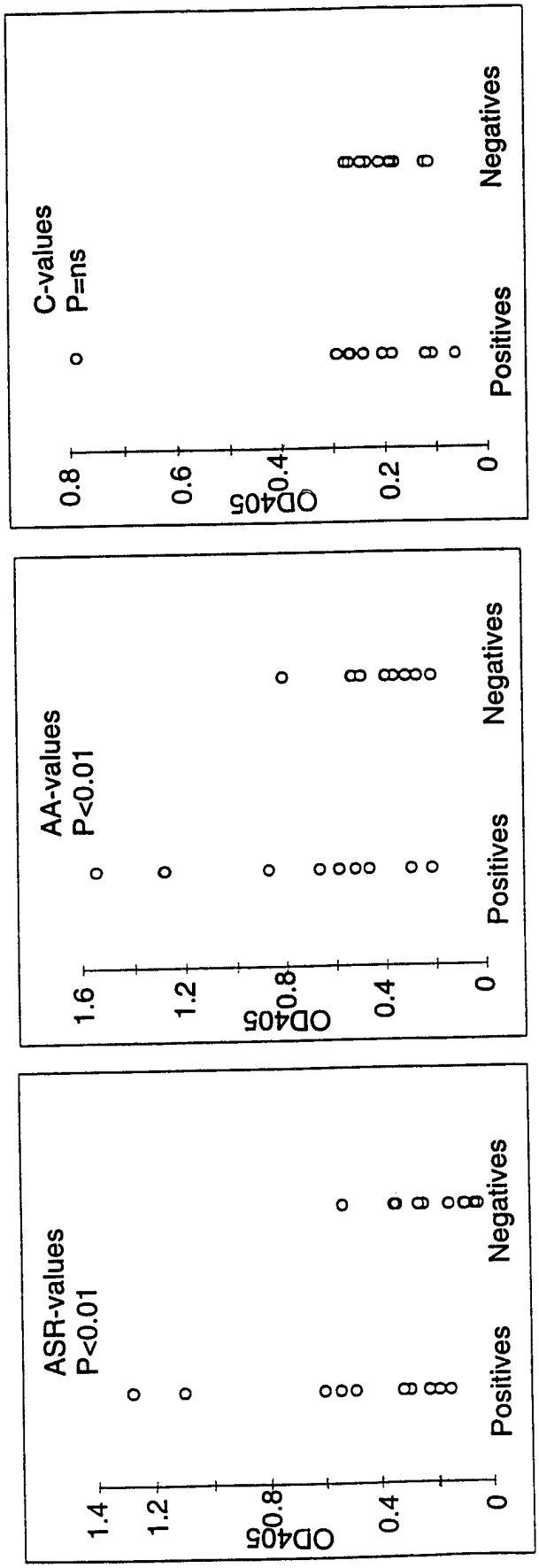


Figure 3

IgA reactivity to albumin-antigen measured by IgA ASR EIA



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00351

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 33/53, G01N 33/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Alcoholism: Clinical and Experimental Research, Volume 20, August 1996, Simon Worrall, et al, "Relationship Between Alcohol Intake and Imunoglobulin A Immunoreactivity with Acetaldehyde-Modified Bovine Serum Albumin" page 836 - page 840 --	1-9
A	Eur. J. Biochem., Volume 240, 1996, Pekka Sillanaukee et al, "Structural characterisation of acetaldehyde adducts formed by a synthetic peptide mimicking the N-terminus of the hemoglobin beta-chain under reducing and nonreducing conditions", page 30 - page 36, see page 35, right column --	1-9

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 June 1998	25.06.98
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00351

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4647654 A (WILLIAM J. KNOWLES ET AL), 3 March 1987 (03.03.87), see examples 7 and 9 --	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SE 98/00351

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4647654 A	03/03/87	AU 594651 B	15/03/90
		AU 3375589 A	17/08/89
		AU 4926085 A	08/05/86
		DE 3586679 A	29/10/92
		DE 3587687 D,T	07/04/94
		DK 130791 A	04/07/91
		DK 165327 B	09/11/92
		DK 167825 B	20/12/93
		DK 494085 A	30/04/86
		EP 0185870 A,B	02/07/86
		SE 0185870 T3	
		EP 0316306 A,B	17/05/89
		SE 0316306 T3	
		FI 84107 B,C	28/06/91
		FI 854187 A	30/04/86
		FI 904226 D	00/00/00
		IE 63731 B	14/06/95
		IE 63768 B	14/06/95
		JP 7023891 B	15/03/95
		JP 7051087 A	28/02/95
		JP 61172064 A	02/08/86
		US 4658022 A	14/04/87
		US 4727036 A	23/02/88