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Class	Subclass
ISSUE CLASSIFICATION	

UTILITY
SERIAL
NUMBER 08/159969

PATENT DATE FEB 20 1996

PATENT
NUMBER

5492812



5492812

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT
08/159,969	11/30/93 RULE 60	435	7.1	1802

APPLICANTS

H. PAUL VOORHEIS, DUBLIN, IRELAND.

CONTINUING DATA***
VERIFIED ^{3/15/94} THIS APPLN IS A CON OF 07/738,778 08/01/91 ABN
CB

FOREIGN/PCT APPLICATIONS***
VERIFIED
none CB

Foreign priority claimed 35 USC 119 conditions met	☐ yes ☒ no ☐ yes ☒ no CB	AS FILED →	STATE OR COUNTRY IEX	SHEETS DRWGS. 1	TOTAL CLAIMS 5	INDEP. CLAIMS 4	FILING FEE RECEIVED \$1,014.00	ATTORNEY'S DOCKET NO. 4697059
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PENNIE & EDMONDS
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NEW YORK, NY 10036-2711

TITLE
DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE BY SCREENING FOR Tau peptides
IN THE BLOOD OF A PATIENT.

U.S. DEPT. of COMM.-Pat. & TM Office-PTO-436L (rev. 10-78)

PARTS OF APPLICATION
FILED SEPARATELY

D. Williams
Applications Examiner

NOTICE OF ALLOWANCE MAILED

PATRICIA A. DUFFY
Assistant Examiner

CLAIMS ALLOWED

Total Claims	Print Claim
2	1

ISSUE FEE

Amount Due	Date Paid
1210.00	10-19-93

TONI R. SCHEINER
SUPERVISORY PATENT EXAMINER
GROUP 1800

DRAWING

Sheets Drwg.	Figs. Drwg.	Print Fig.
12	2	NOV2

ISSUE
BATCH
NUMBER N54

Label
Area

PREPARED FOR ISSUE

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08/159969

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CONTENTS

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3000 in Group 1800

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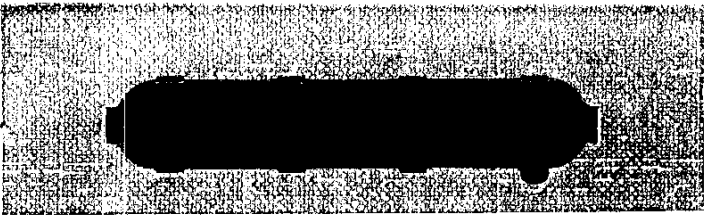
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GROUP 1800

1.	Application _____ papers.	
2.	45 Day Letter (152)	12/21/93
3.	Statement (152)	2/1/94
4.	ACCESS ACKNOWLEDGEMENT	10 MAR 1994
5.	Prel Amdt A	11/30/93
6.	Prel Amdt B	11/30/93
7.	Rej 3mo	4/5/94
8.	Declaration	10-20-94
9.	Disclosure Statement	10/11/94
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14.	Notice of Appeal	6-21-95
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16.	Interview Summary	7-19-95
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18.	90 Day Statement - Done	8/7/95
19.	Ver Waiver	11/20/95
20.	Formal Drawings (2 sheet) set 1	10-22-95
21.	PROCHART FEB 20 1996	
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INTERFERENCE SEARCHED			
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SEARCH NOTES		
	Date	Exmr.
APS DIALOG	4/1/94	CS
reviewed parent including SEA search + print out	3/31/94	CS
consulted C. Tidwell (now in PCT)	1/17/95	DE
updated search	1/10/95	PAD
update	4/13/95	PAD

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POSITION		ID NO.	DATE
CLASSIFIER		10	12-13-93
EXAMINER		38	12/27/93
TYPIST		21	1/27/94
VERIFIER		21	1/27/94
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INDEX OF CLAIMS

Claim		Date			
Final	Original				
1	1	1/2	1/2	1/2	1/2
2	2	1/2	1/2	1/2	1/2
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SYMBOLS
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- (Through numeral) Canceled
+ Restricted
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I Interference
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PATENT NUMBER

ORIGINAL CLASSIFICATION

CLASS

SUBCLASS

435

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APPLICATION SERIAL NUMBER

08/159,969

APPLICANT'S NAME (PLEASE PRINT)

Voorheis, Paul H.

CROSS REFERENCE(S)

CLASS

SUBCLASS
(ONE SUBCLASS PER BLOCK)

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IF REISSUE, ORIGINAL PATENT NUMBER

INTERNATIONAL CLASSIFICATION

G O I N

33/53

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33/543

GROUP
ART UNIT

1802

ASSISTANT EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

PATRICIA A. DUFFY

PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

TOM SCHNEIDER

PTO 270
(REV. 5-91)

ISSUE CLASSIFICATION SLIP

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

Claim	Final	Original	Date
1	✓	✓	1/10/94
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SYMBOLS

- ✓ Rejected
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- (Through numeral) Canceled
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- N Non-elected
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Claim	Final	Original
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US005492812A

United States Patent [19]
Vooheis

[11] Patent Number: 5,492,812
[45] Date of Patent: Feb. 20, 1996

- [54] **DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE BY SCREENING FOR TAU-PEPTIDES IN THE BLOOD OF A PATIENT**
- [75] Inventor: H. Paul Vooheis, Dublin, Ireland
- [73] Assignee: Provost, Fellows and Scholars of Trinity College, Dublin, Ireland
- [21] Appl. No.: 159,969
- [22] Filed: Nov. 30, 1993

Related U.S. Application Data

- [63] Continuation of Ser. No. 738,778, Aug. 1, 1991, abandoned.
- [51] Int. Cl.⁶ G01N 33/53; G01N 33/537; G01N 33/543
- [52] U.S. Cl. 435/7.1; 435/7.92; 435/7.93; 435/7.94; 435/7.95; 436/518; 436/804; 436/811
- [58] Field of Search 435/7.1, 7.2, 7.21, 435/7.92, 975; 436/506, 518, 512, 811, 864, 828, 515, 516, 808

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Iqbal et al., 1989, "Laboratory Diagnostic tests for Alzheimer's disease", *Prog. Clin. Biol. Res.* 317:679-687.

Primary Examiner—Toni R. Scheiner
Assistant Examiner—Patricia A. Duffy
Attorney, Agent, or Firm—Pennie & Edmonds

ABSTRACT

The present invention is directed to methods and kits for diagnosing Alzheimer's disease. The invention is based, in part, on the discovery that proteolytic fragments of the amino and carboxy terminal amino acid residues of tau-proteins are released from the neurofibrillary tangles associated with the disease and can be detected in body fluids outside the brain. The tau-proteins will be purified or chemically synthesized and peptide fragments of the amino terminal and carboxy terminal regions will be obtained proteolytically or synthesized chemically and will be used in generating tau specific antibodies for use in diagnostic kits for the detection of Alzheimer's disease. These diagnostic kits will be used in screening the body fluids of individuals for the presence of tau-peptide fragments. Alternatively, the tau-peptides themselves may be used in diagnostic kits for screening the body fluids of individuals for the presence of circulating autoantibodies.

2 Claims, 2 Drawing Sheets

-37 CCGCCCTCTGTCGACTATCAGGTGAACCTTTGAACCAGG

1 Met Ala Glu Pro Arg Gln Glu Phe Glu Val Met Glu Asp His Ala Gly Thr Tyr Gly Leu Gly Asp Arg Lys Asp Gln Gly Tyr Thr
1 ATG GCT GAG CCC CGC CAG GAG TTC GAA GTG ATG GAA GAT CAC GCT GGG AGG TAC GGG TTT GGG GAC AGG AAA GAT CAG GGG GGC TAC ACC

31 Met His Gln Asp Gln Glu Gly Asp Thr Asp Ala Gly Leu Lys Ala Glu Glu Ala Gly Ile Gly Asp Thr Pro Ser Leu Glu Asp Glu Ala
91 ATG CAC CAA GAC CAA GAG GGT GAC ACC GAC GCT GGC CTG AAA GCT GAA GAA GCA GGC ATT GCA GAC ACC CCC ACC CTG GAA GAC GAA CCT

61 Ala Gly His Val Thr Gln Ala Arg Met Val Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Lys Lys Ala Lys Gly Ala Asp Gly Lys
101 GCT GGT CAC GTG ACC CAA CCT CCC ATG ATG ATG ACT AAA AGC AAA GAC GGG ACT GGA AGC GAT GAC AAA AAA GGC AAG GGG GCT GAT GGC AAA

91 Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro Pro Ala
271 ACG AAG ATC GCC ACA CCG CCG GCA GCA GCC CCT CCA GGC CAG AAG GGC CAG GCC AAC GGC ACC ACC ATT CCA GCA AAA ACC CCG CCC GCT

121 Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser
361 CCA AAG ACA CCA CCC AGC TCT GGT GAA CCT CCA AAA TCA GGG GAT CGC AGC GGC TAC AGC AGC CCC GGC TCC CCA GGC ACT CCC GGC AGC

151 Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser
451 CGC TCC CGC ACC CGC TCC CTT CCA ACC CCA CCC ACC CGG CAG CCC AAG AAG GTG GCA GTG GTC CGT ACT CCA CCC AAG TCG CGG TCT TCC

181 Ala Lys Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His
541 GCC AAG AGC CGC CTG CAG ACA GCC CGC CGC GTG CCC ATG CCA GAC CTG AAG AAT GTC AAG TCC AAG ATC GGC TCC ACT GAG AAC CTG AAG CAC

211 Gln Pro Gly Gly Lys Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His
631 CAG CGC GGA GGC GGG AAG GTG CAA ATA GTC TAC AAA CCA GTT GAC CTG ACC AAG GTG ACC TCC AAG TGT GGC TCA TTA GGC AAC ATC CAT

FIG.1A

241 His Lys Pro Gly Gly Gly Gln Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser Lys Ile Gly Ser Leu Asp Asn
721 CAT AAA CCA CCA GGT GGC CAG CTC GAT CTA AAA TCT CAG AAG CTT GAC TTC AAG GAC AGA GTC CAG TCG AAG ATT CGG TCC CTG GAC AAT

271 Ile Thr His Val Pro Gly Gly Gly Asn Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala Lys Thr Asp His Gly
811 ATC ACC CAC CTC CCT GGC GCA GCA AAT AAA AAG ATT GAA ACC CAC AAG CTG ACC TTC CGC CAG AAG GCC AAA GCC AAG ACA GAC CAC GCG

301 Ala Glu Ile Val Tyr Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Thr Gly Ser Ile Asp Met
901 GCG GAG ATC GTG TAC AAG TCG CCA GTG GTG TCT CGG GAC ACG TCT CCA CGG CAT CTC AGC AAT GTC TCC ACC GGC AGC ATC GAC ATG

331 Val Asp Ser Pro Gln Leu Ala Thr Leu Ala Asp Glu Val Ser Ala Ser Leu Ala Lys Gln Gly Leu ***
991 GTA GAC TCG CCC CAG CTC GGC ACC CTA GCT GAC GAG GTG TCT GCC TCC CTC GGC AAG CAG GGT TTG TGA TCAGGCCCTGG

FIG.1B

DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE BY SCREENING FOR TAU-PEPTIDES IN THE BLOOD OF A PATIENT

This is a continuation, of application Ser. No. 07/738, 778, filed Aug. 1, 1991, now abandoned.

1. FIELD OF THE INVENTION

The present invention is directed to the diagnosis, subtyping and monitoring of Alzheimer's disease. Diagnostic methods and kits are described which utilize monoclonal antibodies, polyclonal antibodies or Fab fragments specific to peptide fragments of the tau family of proteins which are found in the blood or spinal fluid of individuals affected by Alzheimer's disease. Alternatively, tau-peptides can be used to detect circulating autoantibodies in such patients.

The diagnostic Alzheimer's disease detection kits of the present invention can be particularly useful in screening the overall population and identifying those individuals who have Alzheimer's disease as well as subtyping the affected individuals according to which member of the tau family of proteins they express.

2. BACKGROUND OF THE INVENTION

Alzheimer's disease is the most common form of both senile and presenile dementia in the world and is recognized clinically as relentlessly progressive dementia that presents with increasing loss of memory, intellectual function and disturbances in speech (Merritt, 1979, *A Textbook of Neurology*, 6th edition, pp. 484-489 Lea & Febiger, Philadelphia). The disease itself usually has a slow and insidious progress that affects both sexes equally, worldwide. It begins with mildly inappropriate behavior, uncritical statements, irritability, a tendency towards grandiosity, euphoria and deteriorating performance at work; it progresses through deterioration in operational judgement, loss of insight, depression and loss of recent memory; it ends in severe disorientation and confusion, apraxia of gait, generalized rigidity and incontinence (Gilroy & Meyer, 1979, *Medical Neurology*, pp. 175-179 MacMillan Publishing Co.). Alzheimer's disease afflicts an estimated 4 million human beings in the United States alone at a cost of 35 billion dollars a year (Hay & Ernst, 1987, *Am. J. Public Health*, 77:1169-1175). It is found in 10% of the population over the age of 65 and 47% of the population over the age of 85 (Evans et al., 1989, *JAMA*, 262:2551-2556). In addition, the disease is found at much lower levels in the younger age groups, usually beginning at about 30 years of age and even rarely in late childhood (Adams & Victor, 1977, *Principles of Neurology*, pp. 401-407).

The etiology of Alzheimer's disease is unknown. Evidence for a genetic contribution comes from several important observations such as the familial incidence, pedigree analysis, monozygotic and dizygotic twin studies and the association of the disease with Down's syndrome (for review see Baraitser, 1990, *The Genetics of Neurological Disorders*, 2nd edition, pp. 85-88). Nevertheless, this evidence is far from definitive and it is clear that one or more other factors are also required. Elevated concentrations of aluminum have been found in the brains of some patients dying with Alzheimer's disease (Crapper et al., 1976, *Brain*, 99:67-80) and one case report has documented markedly elevated levels of manganese in the tissues of a patient with Alzheimer's disease (Banta & Markesberg, 1977, *Neurol-*

ogy, 27:213-216), which has led to the suggestion that high levels of these metals may be neurotoxic and lead to the development of Alzheimer's disease. It was interesting that the aluminum ions were found to be associated mainly with the nuclear chromatin in brain regions most likely to display neurofibrillary tangles in Alzheimer's disease. However, from a statistical point of view the absolute differences found for the aluminum levels between normal and Alzheimer brains were far from convincing. It has recently been suggested that defects in the transcriptional splicing of mRNA coding for the tau complex of microtubule associated proteins occur (for review see Kosik, 1990, *Curr. Opinion Cell Biol.*, 2:101-104) and/or that inappropriate phosphorylation of these proteins exists (Grundke-Igbak et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:4913-4917; Wolozin & Davies, 1987, *Ann. Neurol.* 22:521-526; Hyman et al., 1988, *Ann. Neurol.*, 23:371-379; Bancher et al., 1989, *Brain Res.*, 477:90-99). Furthermore, reduction in the enzymes involved in the synthesis of acetylcholine has led to the view of Alzheimer's disease as a cholinergic system failure (Danes & Moloney, 1976, *Lancet*, ii:1403-14). However, even if cholinergic neurons are most at risk in Alzheimer's disease, it appears likely that these reductions in enzyme activity are secondary to the degenerative process itself rather than causally related.

2.1 THE DIAGNOSIS OF ALZHEIMER'S DISEASE

The diagnosis of Alzheimer's disease at autopsy is somewhat time-consuming but is definitive. The gross pathological changes are found in the brain, which is underweight, and shows generalized atrophy of both the gray and white matter of the cerebral cortex, particularly in the temporal and frontal lobes, with widening of the sulci and narrowing of the gyri as well as compensatory symmetrical dilation of the third and lateral ventricles (Adams & Victor, 1977, *Principles of Neurology*, pp. 401-407; Merritt, 1979, *A Textbook of Neurology*, 6th edition, Lea & Febiger, Philadelphia, pp. 484-489). The histological changes in the brain are definitive. The most common lesion is the neurofibrillary tangle (Kidd, 1963, *Nature*, 197:192-193; Kidd, 1964, *Brain* 87:307-320), which consists of a tangled mass of paired helical filaments and occasional straight filaments located in the cytoplasm of affected neurons (Oyanagai, 1979, *Adv. Neurol. Sci.*, 18:77-88 (in Japanese) as quoted by Grundke-Iqbal et al., 1985, *Acta Neuropathol.*, 66:52-61). The other characteristic histological lesions are Hirano bodies within nerve cells of certain brain regions, neuritic dystrophy and amyloid angiopathy. In addition, there are prominent argento-philic senile neuritic plaques composed of enlarged, degenerating axonal endings, containing degraded cellular organelles and some paired helical filaments, that surround a core of mainly extracellular amyloid (Merritt, 1979, *A Textbook of Neurology*, 6th edition, Lea & Febiger, Philadelphia, pp. 484-489). Small amounts of these histological changes are found also in the brains of almost all very old humans at autopsy, even if they showed no symptoms of Alzheimer's disease before death (Tomlinson & Henderson, 1976, *Neurobiology of Aging*, p. 183, Terry & Gershon (eds.), Raven, N.Y.; Ball, 1976, *Neuropathol. Appl. Neurobiol.*, 2:395; Ball, 1977, *Acta Neuropathol.*, 37:111-118). However, the number and total amount of these histological changes in brain has been found to correlate well with the severity of the clinical signs and symptoms of Alzheimer's disease (Kidd, 1963, *Nature*, 197:192-193; Terry et al., 1964, *Am. J. Pathol.*, 44:269-297; Roth et al., 1966, *Nature*,

209:109-110; Blessed et al., 1968, *Br. J. Psychiat.*, 114:797-881; Tomlinson et al., 1970, *J. Neurol. Sci.*, 11: 205-242; Wisniewski, 1976, *J. Neurol. Sci.*, 27:173-181).

The diagnosis of Alzheimer's disease during life is more difficult than at autopsy since it depends upon an inexact clinical evaluation. In the middle stages and particularly in the earlier stages of the disease, the diagnosis is a matter of clinical judgement and success is markedly dependent upon the experience and clinical acumen of the attending physician or consultant. In the late stages of the disease however, clinical diagnosis is relatively straight-forward even if it is not completely unequivocal. In all cases, a wide variety of other diseases, presenting at least partially overlapping clinical pictures, must be positively ruled out before a diagnosis of Alzheimer's disease can be made. Usually a patient must be evaluated on a number of occasions over some period of time in order to document the deterioration in intellectual ability and other signs and symptoms before a diagnosis can be attempted with reasonable confidence. The necessity of repeated evaluation by a professional medical practitioner before a firm diagnosis can be made is costly, generates anxiety and can be frustrating to patients and their families. Furthermore, the development of an appropriate therapeutic strategy is greatly hampered by these difficulties in the objective and rapid diagnosis of the disease, particularly in the early stages where even a simple arrest of the progress of the disease would leave the patient with significant intellectual capacity and a reasonable quality of life.

No unequivocal laboratory test specific for Alzheimer's disease has been reported. European patent application No. 391714 discloses a method for the diagnosis of Alzheimer's disease based upon the detection of mature β -amyloid protein, its precursor protein or a fragment of β -amyloid protein in non-neural tissue biopsy using immunoassay techniques. However, this approach is non-specific since it has been recognized that a wide variety of degenerative diseases, lead to the production and deposition of β -amyloid protein in almost all human tissues. Furthermore, even though the gene coding for both the β -amyloid protein (Goldgaber et al., 1987, *Science*, 235:877-80; Tanzi et al., 1987a, *Science*, 235:880-884) and the gene for familial Alzheimer's disease (St. George-Hyslop et al., 1987, *Science*, 325:885-890) have been located on human chromosome 21, it has been shown that the linkage between the two genes is not very close (Van Broedkoven et al., 1987, *Nature*, 329:153-155; Tanzi et al., 1987b, *Nature*, 329:156-157). In addition, it is known that despite some earlier claims, β -amyloid protein forms no part of the paired helical filaments within neurofibrillary tangles (for review see Wischik et al., 1989, *Curr. Opinion Cell Biol.*, 1:115-122). Therefore, even though β -amyloid protein is deposited in many tissues of patients with Alzheimer's disease, it has not been definitively linked to Alzheimer's disease in a manner different to its association with many other degenerative diseases. Thus, the diagnostic usefulness of antibodies directed to mature β -amyloid protein, its precursor or any of its fragments must be considered marginal at best.

2.2 NEUROFIBRILLARY TANGLES AND ALZHEIMER'S DISEASE

It is now known that the only definitive feature of Alzheimer's disease is the presence of grossly increased quantities of neurofibrillary tangles within the affected cortical regions of brain compared to either normal brain from humans of any age or to brain from any disease state other than

Alzheimer's disease itself. However, the neurofibrillary tangles have not heretofore been exploited for use in a diagnostic or screening assay for Alzheimer's disease.

The progression of Alzheimer's disease is characterized by a loss of cortical substance in the brain. This fact has been well documented over many years by many different researchers. The most characteristic lesion in Alzheimer's disease is the presence of paired helical filaments in discrete, randomly interwoven groups or neurofibrillary tangles within affected cortical neurons. In addition, the number and size of these tangles within an affected neuron as well as the total number of tangles and the total amount of constituent tau-protein in an affected brain correlates with the progression and severity of the disease. Therefore, it is believed that as these tangles increase in size and number they must interfere with the physiological function of each cell in which they occur, eventually leading directly to the death and lysis of that cell. Recently, it has been reported that the cytosolic ATP-dependent protease signal protein, ubiquitin, becomes attached to neurofibrillary tangles (Mori et al., 1987, *Science*, 235:1641-1644). Since ubiquitinated proteins are rapidly degraded, this suggests that the affected cell recognizes the tangles as a foreign structure requiring degradation. It is further believed that this mechanism of cortical neuron death is responsible for the progressive loss of cortical substance and, therefore, the loss of intellectual capacity as well as the appearance of the other signs and symptoms of Alzheimer's disease.

The paired helical filaments are composed of an unknown protein of 90,000 molecular weight (Mr) associated with various tau-protein species. A tightly bound helical core is formed between the 90,000 Mr protein and the large middle domain of the tau-protein as well as a region of the C-terminal domain, which contains the imperfect tandem repeat. The structural conformation of the helical core allows the N-terminal and C-terminal domains of the tau-protein to protrude at some angle from the axis of the paired helical filament thereby forming a protease-sensitive coat around the paired helical filaments (Wischik et al., 1988, *Proc. Natl. Acad. Sci. USA.*, 85:4884-4888). The research groups working in this area are directing their efforts towards characterizing the protease-resistant core of the paired helical filaments in order to understand the nature of the stable interaction and the nature of the modification that occurs to tau-proteins in Alzheimer's disease (for reviews, see Wischik et al., 1989, *Curr. Opinion Cell Biol.*, 1:115-122; Kosik, 1990, *Curr. Opinion Cell Biol.*, 2:101-104). This orientation of research effort as well as research directed towards the molecular genetics of the tau-protein are likely to lead to an understanding of the primary cause of Alzheimer's disease.

From the foregoing, it is clear that no one concerned with Alzheimer's disease has realized that its diagnosis ultimately must depend upon the detection and quantitative measurement of the amount of the component substance or substances that constitute these neurofibrillary tangles in victims of the disease. The detection of associated substances that can also be found in other disease states can never be as definitive as the detection of the actual substance building up in affected neurons in Alzheimer's disease causing the eventual death of those cells. What has also not been realized is that the protease stability of the core of the paired helical filaments makes it unlikely that any part of the tau-protein or the 90,000 Mr protein present in this stable core will ever be found outside the brain. The slow destruction of these stable cores, following their release by dead neurons, can be conceived to occur only in situ and as a consequence of

phagocytosis by microglia. The most likely reason for the reluctance or failure to recognize the potential of using components of the neurofibrillary tangles in the diagnosis of Alzheimer's disease relates to the natural abhorrence that a person would feel if required to biopsy the brain of any living human being even in the last stages of the disease in order to measure these substances. The biopsy procedure is extremely invasive and must leave the person so biopsied less than whole. However, the scientific community has not considered the possibility that biopsy might not be necessary in order to detect the substance or substances in question.

In contrast to the relevant literature, the diagnostic approach of the present invention is based on the recognition that the whole of the 200 amino acid N-terminal residues of the various tau-proteins as well as some portion of their 50 amino acid most C-terminal residues will be released when cleaved from the filaments by ubiquitin-recognizing proteases or other proteases during degeneration and rupture of affected neurons. The diagnostic assays of the invention are based on the principle that it is these cleaved latter segments of tau-proteins that can find their way into body fluids outside the brain and whose detection and quantitation are important for diagnostic purposes. Additional support for this last conclusion comes from the observation that other brain-specific, soluble and protease-sensitive proteins find their way into blood and spinal fluid following head trauma (Phillips et al., 1980, *Br. Med. J.*, 281:777-779; Brayne et al., 1982, *Lancet*, ii:1308-9) and various other types of neurological disorders (Thompson et al., 1980, *J. Neurol. Sci.*, 47:241-254; Willson et al., 1980, *Ann. Clin. Biochem.*, 17:110-113).

3. SUMMARY OF THE INVENTION

The present invention relates to methods and kits for diagnosing, subtyping and monitoring Alzheimer's disease. The invention is based, in part, on the discovery that proteolytic fragments of the amino and carboxy terminal amino acid residues of tau-proteins are released from the neurofibrillary tangles associated with the disease and can be detected in body fluids outside the brain.

The generation and purification of tau-proteins and peptide fragments of the amino terminal and carboxy terminal regions of tau-proteins are described. These tau-proteins and fragments thereof may be used to generate monoclonal antibodies, polyclonal antibodies or Fab fragments that, in turn, can be used in immunoassays to detect circulating tau-peptides in suspected individuals. Alternatively, the tau-peptides themselves may be used in immunoassays to detect circulating autoantibodies in such individuals.

3.1 DEFINITIONS

The term tau-protein is defined herein as any one of the proteins that comprise a single constituent part of the tau-complex of proteins.

The tau-complex of proteins is defined as that group of microtubule associated proteins (MAPS) from vertebrate brain that have the following characteristics: (a) co-purify with tubulin during the cycle purification of microtubules, particularly in the absence of glycerol (Asnes & Willson, 1979, *Anal. Biochem.*, 98:64-73; Murphy & Hiebsch, 1979, *Anal. Biochem.*, 96:225-235); (b) separate from tubulin present in the microtubules obtained by the cycle technique when an ice-cold sample of such a preparation is chromatographed on a phosphocellulose column, using 2-(N-morpholino)-ethanesulphonic acid (MES) buffer to remove

tubulin and using MES buffer containing 1M NaCl to remove the tau-complex of proteins (Weingarten et al., 1975, *Proc. Natl. Acad. Sci. USA*, 72:1858-1862; Cleveland et al., 1977, *J. Mol. Biol.*, 116:207-225; Cleveland et al., 1979, *J. Biol. Chem.*, 254:12670-12678); (c) are included in a column of Sepharose 6B when chromatographed on such a matrix and where they migrate as an aggregated species not far from the exclusion volume but have individual unaggregated native molecular masses between 10,000 and 100,000 Mr regardless of axial ratio (Cleveland et al., 1977, *J. Mol. Biol.*, 116:207-225); (d) react with any of a number of antibodies described in the literature as "anti-tau" or with a commercial antibody described as anti-tau; (e) contain within each and every one of their C-terminal domains an imperfect tandem repeat of the type described in the literature (Lewis et al., 1988, *Science*, 242:936-939; Goedert et al., 1989, *EMBO J.*, 8:393-399; Kanai et al., 1989, *J. Cell Biol.*, 109:1173-1184); and (f) are either heat-stable or heat-labile as described by Cleveland et al., 1977, *J. Mol. Biol.*, 116:207-225.

The term "forward sandwich assay" is defined in its most basic form as an assay wherein the sample to be tested containing the tau-peptide reacts with an anti-tau-peptide antibody that has been immobilized upon a solid substrate; then a labeled second anti-tau-peptide antibody is added, which reacts with the tau-peptide at a different site.

The term "reverse sandwich assay" is defined in its most basic form as an assay wherein the labeled anti-tau-peptide antibody and sample to be tested containing the tau-peptide are first incubated together and then added to a second unlabeled anti-tau-peptide antibody that had previously been immobilized upon a solid substrate as in the "forward sandwich assay". Both the labeled and the unlabeled anti-tau-peptide must react with the tau-peptide at a different site.

4. DESCRIPTION OF THE FIGURE

FIG. 1 shows the complete nucleotide (SEQ ID NO: 1) and amino acid sequences (SEQ ID NO: 2) of one form of human tau-protein, reproduced from Goedert et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:4051-4055.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention results from the unexpected discovery of several physiological functions for individual proteins in the tau-complex of proteins that have not heretofore been described and are in addition to the well recognized function of promoting the polymerization of microtubules and/or their stability. This discovery has permitted the development of assays for the individual proteins in the tau-complex of proteins and their subsequent purification with retention of their native configuration and physiological activity. The invention is based, in part, on the discovery that in patients suffering from Alzheimer's disease there is proteolytic fragmentation of the tau-complex of proteins which occurs independently of phosphorylation, such that only the N-terminal domain comprising up to not more than the 200 most N-terminal amino acids or less and the C-terminal domain comprising up to only the 50 most C-terminal amino acids or less of tau will be released from the paired helical filaments, resident in the neurofibrillary tangle or free in the cytoplasm of affected neurons, and make their way into spinal fluid and blood.

This invention provides the first demonstration that any part of the tau-proteins of brain in victims of Alzheimer's disease are also present in body fluids outside the brain itself in concentrations greatly exceeding that found in normal humans that are not victims of Alzheimer's disease. This is achieved by examining a sample of body fluid, preferably blood or spinal fluid, and detecting the presence and quantity of any of the tau-proteins or fragments thereof. The detection and quantitative measurement of any of these tau-proteins or fragments thereof in body fluids in accordance with this invention is useful in confirming a clinical diagnosis of Alzheimer's disease in affected patients and in following the course of the disease. The invention is also useful in monitoring the disease during and subsequent to a period of treatment with agents that are being tested for their ability to stabilize, decrease or prevent the release of tau-proteins or fragments thereof from the brain.

Alternatively, the invention is directed to the production of tau-peptides for use in detecting circulating anti-tau-peptide autoantibodies in body fluids outside the brain in victims of Alzheimer's disease. The detection of circulating anti-tau-peptide autoantibodies in accordance with this invention is useful in confirming a clinical diagnosis of Alzheimer's disease in affected patients and in following the course of the disease.

For purposes of description only, the invention will be described in terms of: (a) generating tau-peptides; (b) generating antibodies that define the tau-peptide; and (c) diagnostic assays and kits for diagnosing subtyping or monitoring Alzheimer's disease.

5.1 PRODUCTION OF TAU-PEPTIDES

5.1.1 ENZYMATIC CLEAVAGE OF PURIFIED TAU-PROTEINS

The production of tau-peptides may be accomplished by isolating the paired helical filaments from human brains of patients who died with Alzheimer's disease and purifying the helical complex containing the tau-protein and the 90,000 Mr protein (see Section 6, *infra*). The tau-protein is associated with the 90,000 Mr protein such that the 200 most amino terminal residues and the 50 most carboxy terminal residues protrude from the helical core of the complex thereby rendering those fragments susceptible to proteolytic enzymes.

The cleavage of the tau-protein into two large fragments of amino acid residues 1 to 151 and 154 to 352 and the dipeptide, Ser-Arg, may be accomplished using either one of the arginine-specific proteolytic enzymes such as thrombin (EC 3.4.13) as described recently (Joly et al., 1989, *J. Cell Biol.*, 109:2289-2294) or clostripain (EC 3.4.4.20). The preferred embodiment uses clostripain because the enzyme is a sulphhydryl enzyme, requiring calcium ions for full activity. Consequently, its activity can be terminated conveniently by the addition of excess iodoacetamide and ethylene glycol-bis(β -aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA). In the case of either protease the concentration of tau-protein is adjusted to 100 μ g/ml before beginning the digestion. Purified clostripain is used at a concentration of 4 international units/ml in the following buffer (50 mM phosphate buffer pH 7.7, 1 mM β -mercaptoethanol, and 1 mM calcium acetate). Soybean trypsin inhibitor (clostripain/inhibitor molar ratio=10/1) is added in order to eliminate any small residual trypsin activity in commercially available preparations of the enzyme. The cleavage reaction is conducted at 37° C. and is terminated by

the addition of a sufficient volume of a 100 mM stock solution EGTA, pH 7.7, containing 100 mM iodoacetamide to give a final concentration of 2 mM EGTA in the protease reaction mixture. The exact time required for proteolysis varies with each tau-protein and markedly depends upon the batch of clostripain used. Therefore, the optimum time for a single cleavage must be determined for each combination of clostripain batch and tau-protein used. The time course of the cleavage reaction is determined by conducting the reaction as described above, but on a 20 μ l volume and assaying the cleavage reaction by subjecting samples at various times to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) according to the method of Laemmli, (*Nature*, 1970, 227:680-684) as modified by Studier, *Sci.*, 176:367-376 (1972) using a 10% (w/v) polyacrylamide resolving gel followed by Serva blue staining and destaining in the usual manner of anyone familiar with the art.

The two large tau-peptide fragments resulting from either thrombin or clostripain proteolysis may be further cleaved by digestion with trypsin, which cleaves on the carboxy terminus of lysine or arginine residues. See for example, the techniques described in *Current Protocols in Molecular Biology*, Ausubel et al., Green Publish. Assoc. & Wiley Interscience, Ch. 10.

The sequence of tau-peptides derived from proteolytic digestion may be identified using the Edman degradation method of protein sequencing. This method sequentially removes one amino acid residue at a time from the amino terminal end of a peptide for subsequent sequence identification by chromatographic procedures. See for example, the techniques described in Konigsberg and Steinman, 1977, *Strategy and Methods of Sequence Analysis*, in Neurath and Hill (eds.), *The Proteins* (3rd ed.) Vol. 3, pp. 1-178, Academic Press. In addition, sequence analysis of tau-peptides may be accelerated by using an automated liquid phase amino acid sequenator following recently described techniques (Hewick et al., 1981, *J. Biol. Chem.*, 256:7990-7997; Stein and Undefried, 1984, *Analy. Chem.*, 136:7-23), thereby allowing for the analysis of picomolar quantities of tau-peptides containing up to 50 amino acid residues in length.

5.1.2 CHEMICAL SYNTHESIS OF TAU-PEPTIDES

Tau-peptides can also be produced by chemical synthesis of the amino acid sequence of a tau-protein (Goedert et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:4051-4055), as predicted from the cloning and sequencing of a cDNA coding for a tau-protein. This tau-protein sequence information may be utilized to predict the appropriate amino and carboxy terminal tau-peptides to be chemically synthesized using standard peptide synthesis methods known in the art. These methods include a solid-phase method devised by R. Bruce Merrifield, (Erickson and Merrifield, "Solid-Phase Peptide Synthesis", in *The Proteins*, Volume 2, H. Neurath & R. Hill (eds.) Academic Press, Inc., New York pp. 255-257; Merrifield, 1986, "Solid phase synthesis", *Science*, 242:341-347). In the solid-phase method, amino acids are added stepwise to a growing peptide chain that is linked to an insoluble matrix, such as polystyrene beads. A major advantage of this method is that the desired product at each stage is bound to beads that can be rapidly filtered and washed and thus the need to purify intermediates is obviated. All of the reactions are carried out in a single vessel, which eliminates losses due to repeated transfers of products. This solid phase method of chemical peptide synthesis can

readily be automated making it feasible to routinely synthesize peptides containing about 50 residues in good yield and purity (Stewart and Young, 1984, *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chemical Co.; Tam et al., 1983, *J. Am. Chem. Soc.*, 105:6442). For example, tau-peptides corresponding to amino acid residues 1 to 30 and 331 to 352 as depicted in FIG. 1 could be synthesized.

5.1.3 CLONING AND EXPRESSION OF RECOMBINANT

TAU-PROTEINS AND/OR TAU-PEPTIDES

The production of tau-peptides can further be achieved by recombinant DNA technology. For example, appropriate tau nucleotide coding sequences may be synthesized, cloned and expressed in appropriate host cells. Since the DNA sequence encoding for a tau-protein is known (Goedert et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:4051-4055), DNA probes may be synthesized by standard methods known in the art to screen cDNA libraries prepared from brain tissue of Alzheimer's disease patients for the specific tau-protein cDNA's. These DNA probes can further be used to isolate the entire family of tau-protein genes from these cDNA libraries using methods which are well known to those skilled in the art. See, for example, the techniques described in Maniatis et al., 1982, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y., Chapter 7.

The polymerase chain reaction (PCR) technique can be utilized to amplify the individual members of the tau family for subsequent cloning and expression of tau-protein cDNAs (e.g., see U.S. Pat. Nos. 4,683,202; 4,683,195; 4,889,818; Gyllenstein et al., 1988, *Proc. Nat'l Acad. Sci. USA*, 85:7652-7656; Ochman et al., 1988, *Genetics*, 120:621-623; Triglia et al., 1988, *Nucl. Acids. Res.*, 16:8156; Frohman et al., 1988, *Proc. Nat'l Acad. Sci. USA*, 85:8998-9002; Loh et al., 1989, *Science*, 243:217-220).

Methods which are well known to those skilled in the art can be used to construct expression vectors containing tau-proteins or fragments thereof coding sequences and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., 1982, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y., Chapter 12.

A variety of host-expression vector systems may be utilized to express tau-proteins or fragments thereof. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing a coding sequence for a tau-protein or fragment thereof; yeast transformed with recombinant yeast expression vectors containing a coding sequence for a tau-protein or fragment thereof; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing a coding sequence for a tau-protein or fragment thereof; or animal cell systems infected with recombinant virus expression vectors (e.g., adenovirus, vaccinia virus) containing a coding sequence for a tau-protein or fragment thereof.

The expression elements of these vectors vary in their strength and specificities. Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For

example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when cloning in mammalian cell systems, promoters such as the adenovirus late promoter or the vaccinia virus 7.5K promoter may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted coding sequence for a tau-protein or fragment thereof.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, *Current Protocols in Molecular Biology*, Vol. 2, 1988, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience Ch. 13; Grant et al., 1987, *Expression and Secretion Vectors for Yeast*, in *Methods in Enzymology*, Eds. Wu & Grossman, 1987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, *DNA Cloning*, Vol. II, IRL Press, Wash., D.C. Ch.3; and Bitter, 1987, *Heterologous Gene Expression in Yeast*, *Methods in Enzymology*, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and *The Molecular Biology of the Yeast Saccharomyces*, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II. For complementation assays in yeast, cDNAs for tau-proteins or fragments thereof may be cloned into yeast episomal plasmids (YEep) which replicate autonomously in yeast due to the presence of the yeast 2 μ circle. The tau-protein or fragment thereof sequence may be cloned behind either a constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL (*Cloning in Yeast*, Chpt. 3, R. Rothstein In; *DNA Cloning Vol. 11, A Practical Approach*, Ed. DM Glover, 1986, IRL Press, Wash., D.C.). Constructs may contain the 5' and 3' non-translated regions of a cognate tau-protein mRNA or those corresponding to a yeast gene. YEep plasmids transform at high efficiency and the plasmids are extremely stable. Alternatively vectors may be used which promote integration of foreign DNA sequences into the yeast chromosome.

A particularly good expression system which could be used to express tau-proteins or fragments thereof is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The tau-protein or fragment thereof coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the polyhedrin gene results in production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (e.g., see Smith et al., 1983, *J. Biol.*, 46:586; Smith, U.S. Pat. No. 4,215,051).

In cases where an adenovirus is used as an expression vector, the tau-protein or fragment thereof coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vivo or in vitro recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the tau-protein or fragment thereof in infected hosts. (e.g., See Logan & Shenk, 1984, *Proc. Natl. Acad. Sci., (USA)* 81:3655-3659). Alternatively, the vaccinia 7.5K promoter may be used. (e.g., see Mackett

et al., 1982, *Proc. Natl. Acad. Sci., (USA)* 79:7415-7419; Mackett et al., 1984, *J. Virol.*, 49:857-864; Panicali et al., 1982, *Proc. Natl. Acad. Sci.*, 79: 4927-4931).

Specific initiation signals may also be required for efficient translation of the inserted tau-protein or fragment thereof coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where the entire tau-protein genome, including its own initiation codon and adjacent sequences, are inserted into the appropriate expression vectors, no additional translational control signals may be needed. However, in cases where only a portion of the tau-protein coding sequence is inserted, exogenous translational control signals, including the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the tau-protein or fragment thereof coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bitter et al., 1987, *Methods in Enzymol.*, 153:516-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression driven by certain promoters can be elevated in the presence of certain inducers, (e.g., zinc and cadmium ions for metallothionein promoters). Therefore, expression of the genetically engineered tau-protein or fragment thereof may be controlled. This is important if the protein product of the cloned foreign gene is lethal to host cells. Furthermore, modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

The host cells which contain the tau-protein or fragment thereof coding sequence and which express the biologically active tau-protein or fragment thereof gene product may be identified by at least four general approaches: (a) DNA-DNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by expression of tau-protein mRNA transcripts in host cells; and (d) detection of tau-protein gene products as measured by immunoassays or by its biological activity.

In the first approach, the presence of the tau-protein or fragment thereof coding sequence inserted in the expression vector can be detected by DNA-DNA hybridization using probes comprising nucleotide sequences that are homologous to the tau-protein coding sequence or particular portions thereof substantially as described recently (Goedert et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:4051-4055).

In the second approach, the recombinant expression vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the tau-protein or fragment thereof coding sequence is inserted within a marker gene sequence of the vector, recombinants containing the tau-protein or fragment thereof coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed in

tandem with the tau-protein or fragment thereof coding sequence under the control of the same or different promoter used to control the expression of the tau coding sequence. Expression of the marker in response to induction or selection indicates expression of the tau-protein coding sequence.

In the third approach, transcriptional activity for the tau-protein or fragment thereof coding region can be assessed by hybridization assays. For example, RNA can be isolated and analyzed by Northern blot using a probe homologous to the tau-protein or fragment thereof coding sequence or particular portions thereof substantially as described (Goedert et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:4051-4055). Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of the tau-protein or fragment thereof product can be assessed immunologically, for example by Western blots, immunoassays such as radioimmunoprecipitation, enzyme-linked immunoassays and the like.

Once a recombinant that expresses a tau-protein or fragment thereof is identified, the gene product should be analyzed. This can be achieved by assays based on the physical, immunological or functional properties of the product.

A tau-protein or fragment thereof should be immunoreactive whether it results from the expression of the entire gene sequence, a portion of the gene sequence or from two or more gene sequences which are ligated to direct the production of chimeric proteins. This reactivity may be demonstrated by standard immunological techniques, such as radioimmunoprecipitation, radioimmune competition, or immunoblots.

5.2 GENERATION OF ANTIBODIES THAT DEFINE TAU-PEPTIDES

Various procedures known in the art may be used for the production of antibodies to epitopes of the tau-protein. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and an Fab expression library. For the production of antibodies, various host animals may be immunized by injection with a particular tau-protein, or a synthetic tau-peptide, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

Monoclonal antibodies to peptides of tau may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, (*Nature*, 1975, 256:495-497), the more recent human B-cell hybridoma technique (Kosbor et al., 1983, *Immunology Today*, 4:72) and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention monoclonal antibodies specific to tau-peptides may be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human

antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, *Proc. Natl. Acad. Sci.*, 80:2026-2030) or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, in, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.*, 81:6851-6855; Neuberger et al., 1984, *Nature*, 312:604-608; Takeda et al., 1985, *Nature*, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce tau-peptide-specific single chain antibodies.

An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity to tau-peptides.

Antibody fragments which contain specific binding sites of tau-peptides may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments.

5.3 DIAGNOSTIC ASSAYS AND KITS FOR ALZHEIMER'S DISEASE

Yet another purpose of the present invention is to provide reagents for use in diagnostic assays for the detection of tau-peptides from individuals suffering from Alzheimer's disease.

In one mode of this embodiment, tau-peptides of the present invention may be used as antigens in immunoassays for the detection of those individuals suffering from Alzheimer's disease. The proteins, polypeptides and peptides of the present invention may be used in any immunoassay system known in the art including, but not limited to: radioimmunoassays, enzyme-linked immunosorbent assay, "sandwich" assays, precipitin reactions, gel diffusion immunodiffusion assays, agglutination assays, fluorescent immunoassays, protein A immunoassays and immunoelectrophoresis assays, to name but a few. U.S. Pat. No. 4,629,783 and patents cited therein also describe suitable assays.

According to the present invention, monoclonal or polyclonal antibodies produced to various forms of the tau-protein, can be used in an immunoassay on samples of blood, spinal fluid or other body fluid to diagnose patients with Alzheimer's disease.

In practicing the invention, a sample of blood is removed from the patient by venesection and placed in contact with an anticoagulant such as EDTA, mixed, centrifuged at 600 g for 10 min and the plasma removed as is common in the art or a sample of spinal fluid is removed from the patient by lumbar puncture.

The antibodies described in Section 5.2 supra may be used as the basic reagents in a number of different immunoassays to determine the presence of a particular tau-peptide in a sample of blood or spinal fluid. Generally speaking, the antibodies can be employed in any type of immunoassay,

whether qualitative or quantitative. This includes both the two-site sandwich assay and the single site immunoassay of the non-competitive type, as well as in traditional competitive binding assays.

Particularly preferred, for ease of detection, and its quantitative nature, is the sandwich or double antibody assay, of which a number of variations exist, all of which are intended to be encompassed by the present invention.

For example, in a typical forward sandwich assay, unlabeled antibody is immobilized on a solid substrate, e.g., microtiter plate wells, and the sample to be tested is brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen binary complex, a second antibody, labelled with a reporter molecule capable of inducing a detectable signal, is then added and incubation is continued allowing sufficient time for binding with the antigen at a different site and the formation of a ternary complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal, which may be quantitated by comparison with a control sample containing known amounts of antigen. Variations on the forward sandwich assay include the simultaneous assay, in which both sample and antibody are added simultaneously to the bound antibody, or a reverse sandwich assay in which the labelled antibody and sample to be tested are first combined, incubated and added to the unlabelled surface bound antibody. These techniques are well known to those skilled in the art, and the possibility of minor variations will be readily apparent. As used herein, "sandwich assay" is intended to encompass all variations on the basic two-site technique.

For the sandwich assays of the present invention, the only limiting factor is that both antibodies have different binding specificities for the tau-peptide. Thus, a number of possible combinations are possible.

As a more specific example, in a typical forward sandwich assay, a primary antibody is either covalently or passively bound to a solid support. The solid surface is usually glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinylchloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surfaces suitable for conducting an immunoassay. The binding processes are well known in the art. Following binding, the solid phase-antibody complex is washed in preparation for the test sample. An aliquot of the body fluid containing the tau-peptide to be tested is then added to the solid phase complex and incubated at 25° C. for a period of time sufficient to allow binding of any tau-peptide present to the antibody specific for tau-peptide. The second antibody is then added to the solid phase complex and incubated at 25° C. for an additional period of time sufficient to allow the second antibody to bind to the primary antibody-antigen solid phase complex. The second antibody is linked to a reporter molecule, the visible signal of which is used to indicate the binding of the second antibody to any antigen in the sample. By "reporter molecule", as used in the present specification is meant a molecule which by its chemical nature, provides an analytically detectable signal which allows the detection of antigen-bound antibody. Detection must be at least relatively quantifiable, to allow determination of the amount of antigen in the sample, this may be calculated in absolute terms, or may be done in comparison with a standard (or series of standards) containing a known normal level of antigen.

The most commonly used reporter molecules in this type of assay are either enzymes or fluorophores. In the case of

an enzyme immunoassay an enzyme is conjugated to the second antibody, often by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are well known to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase and alkaline phosphatase, among others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine or toluidine are commonly used. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-tau-peptide complex and allowed to bind to the complex, then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the tertiary complex of antibody-antigen-labeled antibody. The substrate reacts with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an evaluation of the amount of antigen which is present in the serum sample.

Alternately, fluorescent compounds, such as fluorescein or rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic longer wavelength. The emission appears as a characteristic color visually detectable with a light microscope. As in the enzyme immunoassay (EIA), the fluorescent-labelled antibody is allowed to bind to the first antibody-tau-peptide complex. After washing the unbound reagent, the remaining ternary complex is then exposed to light of the appropriate wavelength, and the fluorescence observed indicates the presence of the antigen. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent or bioluminescent molecules may also be employed. It will be readily apparent to the skilled artisan how to vary the procedure to suit the required use.

Alternatively, the sample to be tested either human blood or spinal fluid containing the tau-peptide may be used in a single site immunoassay wherein it is adhered to a solid substrate either covalently or noncovalently. An unlabeled anti-tau-peptide antibody is brought into contact with the sample bound on the solid substrate. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen binary complex a second antibody, labelled with a reporter molecule capable of inducing a detectable signal, is then added and incubation is continued allowing sufficient time for the formation of a ternary complex of antigen-antibody-labeled antibody. For the single site immunoassay, the second antibody may be a general antibody (i.e., xenogeneic antibody to immunoglobulin, particularly anti-(IgM and IgG) linked to a reporter molecule) that is capable of binding an antibody that is specific for the tau-peptide of interest.

Alternatively, the purified tau-proteins or fragments thereof, described in Section 5.1, supra, may be used in a diagnostic assay to detect circulating anti-tau-peptide autoantibodies wherein they are adhered to a solid support

either covalently or non-covalently. The sample, either human blood or spinal fluid diluted in an appropriately buffered medium, is added to the solid support and a sufficient time is allowed for complex formation between the tau-proteins or fragments thereof and any cognate autoantibodies in the sample. The supernatant is removed and the solid support is washed to remove any non-specifically bound proteins. To the complex may be added xenogeneic antisera to human immunoglobulin, particularly anti-(human Ig M and Ig G) in an appropriately buffered medium. The xenogeneic antisera will normally be labeled with a reporter molecule as described above for the forward sandwich assay. Instead of xenogeneic antisera, proteins specific for the immune complex may be employed, e.g., *S. aureus* protein A. The label may then be detected as described above for the forward sandwich assay.

6. EXAMPLE: PRODUCTION AND PURIFICATION OF IMMUNOGENS FOR ANTIBODY PRODUCTION

6.1. PURIFICATION OF MICROTUBULES FROM BOVINE BRAIN BY THE CYCLE PROCEDURE AS A SOURCE OF NATIVE, PHYSIOLOGICALLY ACTIVE MICROTUBULE ASSOCIATED PROTEINS

The cycle purification of microtubules from bovine brain was performed in the presence of guanosine triphosphate and low concentrations of magnesium ions in order to obtain high ratios of microtubule associated proteins to tubulin. This cycle-purification was carried out in five separate steps according to a modification of the method described by Asnes & Wilson, (1979, *Anal. Biochem.*, 98: 64-73).

Step (1) Transport & Dissection: Eight bovine brains (approximately 350 gram (g) each) were removed, cut into pieces, placed in ice-cold transport buffer [20 mM phosphoric acid (adjusted to pH 6.75 with 2M NaOH) 100 mM sodium glutamate, 300 mM sucrose] containing ice cubes made from transport buffer and transported in an ice bucket from the abattoir to the laboratory. The meninges, superficial blood vessels and the majority of the white matter was removed and discarded. The remaining brain tissue (approximately 1200 g) was drained and weighed.

Step (2) Homogenization: The selected brain tissue was dispersed using two separate maneuvers sequentially. The weighed brain tissue from step (1) was passed once through a domestic electric mincer and added to homogenization buffer [200 mM phosphoric acid (adjusted to pH 6.75 with 2M NaOH), 100 mM sodium glutamate, 300 mM sucrose, 4 mM β -mercaptoethanol, 0.3 mM phenylmethanesulphonyl fluoride (PMSF) (stock solution, 100 mM in dimethylformamide (DMF)), 0.3 mM N α -p-tosyl-L-lysine chloromethylketone (TLCK) (prepared in PMSF stock solution), 50 μ g/ml leupeptin, few crystals of catalase] (at 0° C. and at a concentration of 1.5 ml buffer/g tissue). The material was dispersed by gentle stirring with a glass rod. The resulting suspension was then homogenized in three batches with two complete strokes (up and down twice for each batch separately) of a motor driven 1 liter (L) glass (2.2 inch diameter x 18 inch long) Potter—Elvehjem homogenizer fitted with a 2 inch long teflon pestle (0.005 inch clearance) at 0° C.

Step (3) First Cycle: Approximately 3000 ml of the homogenate was added to twelve 250 ml plastic centrifuge tubes and centrifuged at 27,880 g (13,000 rpm in two Sorval RC5B preparative centrifuges containing two GSA rotors precooled to 3° C.) for 53 minutes at 0°-4° C. The super-

natants were carefully removed, combined and cofactor buffer [2.5 mM GTP, .50 mM $MgCl_2$, 1.0 mM EGTA (10xstock solution adjusted to pH 6.75 at 0° C. with NaOH)] prepared in homogenization buffer was added. The solution was mixed and then approximately 1L portions together with a glass thermometer was placed in separate polyethylene sleeves (10 inchx14 inch) and heat sealed. The loaded sleeves were gently agitated in a 50° C. water bath until the temperature of their contents reached 30° C. whereupon they were immediately transferred to a 30° C. water bath where the incubation continued without further agitation for the remainder of a total period of 30 minutes. Assembly was followed viscometrically using calibrated 100-size Cannon Ubbelohde glass viscometers. Approximately 2400 ml of the assembled extract was transferred to 12 clean 250 ml plastic centrifuge jars and centrifuged at 27,880 g (13,000 rpm in two Sorval RC5B preparative centrifuges containing two Sorval GSA rotors prewarmed to 30° C.) for 42 minutes at 30° C. The supernatant was carefully removed with an aspirator and its volume was measured, recorded and discarded.

Step (4) Second Cycle: The final pellet from the first cycle was resuspended in ice-cold homogenization buffer without sucrose (10% of volume of the supernatant from the first cycle) using a precooled small Dounce homogenizer. Approximately 220 ml of the resuspended pellet was incubated at 0° C. for 30 minutes, added to six 36 ml plastic centrifuge tubes, and centrifuged at 39,000 g (14,627 rpm in a Sorval OTD centrifuge containing a Sorval AH-627 rotor precooled to 3° C.) for 40 minutes at 0° to 4° C. The supernatant was carefully transferred to a 1 L glass conical flask and cofactor buffer was added. The solution was mixed and gently swirled in the 50° C. water bath until the temperature reached 30° C. The flask was then immediately transferred to a 30° C. water bath where the incubation continued without further agitation for a total period of only 10 minutes. The extract was transferred to six 36 ml plastic centrifuge tubes and centrifuged at 39,000 g (14,726 rpm in a Sorval OTD ultracentrifuge containing a Sorval AH-627 rotor) for 30 minutes at 30° C. The supernatant was removed carefully with an aspirator and its volume was measured and discarded.

Step (5) Third Cycle—The final pellet from the second cycle was resuspended in ice-cold homogenization buffer without sucrose (40% of the volume of the supernatant from the second cycle) using a clean precooled small Dounce homogenizer. Approximately 90 ml of resuspended pellet was incubated at 0° C. for 30 minutes, transferred to three 36 ml tubes, and centrifuged at 39,000 g (14,726 rpm in a Sorval OTD ultracentrifuge containing a Sorval AH-627 rotor with 36 ml buckets) for 40 minutes at 0° to 4° C. Approximately 80 ml of supernatant was carefully transferred to a 500 ml glass conical flask and cofactor buffer was added. The solution was mixed and gently swirled in the 50° C. water bath until the temperature of the contents of the flask reached 30° C. Then the flask was immediately transferred to the 30° C. water bath where the incubation continued without further agitation for a total period of 20 minutes. The warm extract was transferred to six 17 ml

plastic centrifuge tubes and centrifuged at 39,000 g (Sorval PTD centrifuge containing a Sorval AH-627 rotor with 17 ml buckets) for 40 minutes at 30° C. The supernatant was removed carefully with an aspirator and discarded. Each tube contained a pellet of approximately 140 mg of microtubule (MT) proteins (approximately 50% tubulin and 50% MAPS) and was stored at -70° C.

6.2. ISOLATION OF THE TAU CLASS OF MICROTUBULE ASSOCIATED PROTEINS FROM CYCLE PURIFIED MICROTUBULES

The total MAPS fraction of cycle purified microtubules was separated from tubulin by a modification of the method of Weingarten, (1975, *Proc. Natl. Acad. Sci. USA*, 72:1858-1862). A pellet of microtubules from the cycle purification method was resuspended using a small Dounce homogenizer in MES buffer (0.1M MES; 1 mM EGTA; 0.5 mM $MgCl_2$ and 1 mM β -mercaptoethanol) so that the resulting solution had an absorbance at 280 nm of 2.0-2.5 and then depolymerized by incubation for 30 min at 0° C. The resulting solution was centrifuged at 100,000 g for 1 hour and the pellet discarded. The supernatant was filtered through a 0.2 μ milipore filter and applied to a phosphocellulose column (1.1 cm diameterx2.6 cm length, 2.5 ml bed volume) that had previously been equilibrated in MES buffer. Tubulin was eluted with MES buffer in the void volume of the column and when the absorbance of the eluate had returned to the baseline value, the total MAPS fraction was eluted (either 0.1 or 0.2 ml fractions) with MES buffer containing 1.0M NaCl and collected as a single complex peak of UV-absorbing material that contained multiple proteins.

The total MAPS fraction eluted from the phosphocellulose column was immediately desalted using the rapid Sephadex G-25 centrifugation method of Penefsky (*J. Biol. Chem.*, 1977, 252:2891-2899). The desalted total MAPS fraction was stirred at 0° C. and solid ammonium sulphate added until its concentration reached 35% (w/v). The stirring was continued for 30 minutes and then the suspension was centrifuged at 20,000 g for 15 minutes. The supernatant was removed and again stirred at 0° C. and more solid ammonium sulphate added until its concentration reached 45% (w/v). After a further 15 minute stirring the suspension was again centrifuged at 20,000 g for 15 minutes and the supernatant discarded. The pellet from this last centrifugation step was dissolved in Mes buffer and applied to a column of hydroxyapatite (1 cm diax10 cm length) and eluted with Mes buffer. The fractions containing only four closely spaced peptides of apparent Mr 45,000 to 65,000 by SDS-PAGE were combined. This total eluted tau fraction was reduced to a volume of 1.0 ml with a sartorius collodion membrane operated in water under reduced pressure, according to the instructions of the manufacturer, and then divided into 0.1 ml portions in 0.5 ml plastic minifuge tubes and stored at -20 C.

The invention is not limited to the embodiments described herein and may be modified or varied without departing from the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 2	
(2) INFORMATION FOR SEQ ID NO:1:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 1107 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
(i i) MOLECULE TYPE: DNA (genomic)	
(i x) FEATURE:	
(A) NAME/KEY: CDS	
(B) LOCATION: 38..1092	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:	
CCGCCTCTGT CGACTATCAG GTGAAC TTG AACCAGG ATG GCT GAG CCC CGC CAG	55
Met Ala Glu Pro Arg Gln	
1 5	
GAG TTC GAA GTG ATG GAA GAT CAC GCT GGG ACG TAC GGG TTG GGG GAC	103
Glu Phe Glu Val Met Glu Asp His Ala Gly Thr Tyr Gly Leu Gly Asp	
10 15 20	
AGG AAA GAT CAG GGG GGC TAC ACC ATG CAC CAA GAC CAA GAG GGT GAC	151
Arg Lys Asp Glu Gly Gly Tyr Thr Met His Glu Asp Gln Glu Gly Asp	
25 30 35	
ACG GAC GCT GGC CTG AAA GCT GAA GAA GCA GGC ATT GGA GAC ACC CCC	199
Thr Asp Ala Gly Leu Lys Ala Glu Glu Ala Gly Ile Gly Asp Thr Pro	
40 45 50	
AGC CTG GAA GAC GAA GCT GCT GGT CAC GTG ACC CAA GCT CGC ATG GTC	247
Ser Leu Glu Asp Glu Ala Ala Gly His Val Thr Gln Ala Arg Met Val	
55 60 65 70	
AGT AAA AGC AAA GAC GGG ACT GGA AGC GAT GAC AAA AAA GCC AAG GGG	295
Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Asp Lys Lys Ala Lys Gly	
75 80 85	
GCT GAT GGT AAA ACG AAG ATC GCC ACA CCG CGG GGA GCA GCC CCT CCA	343
Ala Asp Gly Lys Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro	
90 95 100	
GGC CAG AAG GGC CAG GCC AAC GCC ACC AGG ATT CCA GCA AAA ACC CCG	391
Gly Glu Lys Gly Glu Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro	
105 110 115	
CCC GCT CCA AAG ACA CCA CCC AGC TCT GGT GAA CCT CCA AAA TCA GGG	439
Pro Ala Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly	
120 125 130	
GAT CGC AGC GGC TAC AGC AGC CCC GGC TCC CCA GGC ACT CCC GGC AGC	487
Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser	
135 140 145 150	
CGC TCC CGC ACC CCG TCC CTT CCA ACC CCA CCC ACC CGG GAG CCC AAG	535
Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys	
155 160 165	
AAG GTG GCA GTG GTC CGT ACT CCA CCC AAG TCG CCG TCT TCC GCC AAG	583
Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser Ala Lys	
170 175 180	
AGC CGC CTG CAG ACA GCC CCC GTG CCC ATG CCA GAC CTG AAG AAT GTC	631
Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val	
185 190 195	
AAG TCC AAG ATC GGC TCC ACT GAG AAC CTG AAG CAC CAG CCG GGA GGC	679
Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly	
200 205 210	
GGG AAG GTG CAA ATA GTC TAC AAA CCA GTT GAC CTG AGC AAG GTG ACC	727
Gly Lys Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr	
215 220 225 230	
TCC AAG TGT GGC TCA TTA GGC AAC ATC CAT CAT AAA CCA GGA GGT GGC	775

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21

22

-continued

Ser	Lys	Cys	Gly	Ser	Leu	Gly	Asn	Ile	His	His	Lys	Pro	Gly	Gly	Gly	
				235					240					245		
CAG	GTG	GAA	GTA	AAA	TCT	GAG	AAG	CTT	GAC	TTC	AAG	GAC	AGA	GTC	CAG	823
Gln	Val	Glu	Val	Lys	Ser	Glu	Lys	Leu	Asp	Phe	Lys	Asp	Arg	Val	Gln	
			250					255					260			
TCG	AAG	ATT	GGG	TCC	CTG	GAC	AAT	ATC	ACC	CAC	GTC	CCT	GGC	GGA	GGA	871
Ser	Lys	Ile	Gly	Ser	Leu	Asp	Asn	Ile	Thr	His	Val	Pro	Gly	Gly	Gly	
		265					270					275				
AAT	AAA	AAG	ATT	GAA	ACC	CAC	AAG	CTG	ACC	TTC	CGC	GAG	AAC	GCC	AAA	919
Asn	Lys	Lys	Ile	Glu	Thr	His	Lys	Leu	Thr	Phe	Arg	Glu	Asn	Ala	Lys	
	280					285					290					
GCC	AAG	ACA	GAC	CAC	GGG	GCG	GAG	ATC	GTG	TAC	AAG	TCG	CCA	GTG	GTG	967
Ala	Lys	Thr	Asp	His		Gly	Ala	Glu	Ile	Val	Tyr	Lys	Ser	Pro	Val	
	295				300					305					310	
TCT	GGG	GAC	ACG	TCT	CCA	CGG	CAT	CTC	AGC	AAT	GTC	TCC	TCC	ACC	GGC	1015
Ser	Gly	Asp	Thr	Ser	Pro	Arg	His	Leu	Ser	Asn	Val	Ser	Ser	Thr	Gly	
			315					320						325		
AGC	ATC	GAC	ATG	GTA	GAC	TCG	CCC	CAG	CTC	GCC	ACG	CTA	GCT	GAC	GAG	1063
Ser	Ile	Asp	Met	Val	Asp	Ser	Pro	Gln	Leu	Ala	Thr	Leu	Ala	Asp	Glu	
			330					335					340			
GTG	TCT	GCC	TCC	CTG	GCC	AAG	CAG	GGT	TG	TGATCAGGCC	CCTGG					1107
Val	Ser	Ala	Ser	Leu	Ala	Lys	Gln	Gly								
		345					350									

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ala	Glu	Pro	Arg	Gln	Glu	Phe	Glu	Val	Met	Glu	Asp	His	Ala	Gly	
1				5					10					15		
Thr	Tyr	Gly	Leu	Gly	Asp	Arg	Lys	Asp	Gln	Gly	Gly	Tyr	Thr	Met	His	
		20						25					30			
Gln	Asp	Gln	Glu	Gly	Asp	Thr	Asp	Ala	Gly	Leu	Lys	Ala	Glu	Glu	Ala	
		35					40					45				
Gly	Ile	Gly	Asp	Thr	Pro	Ser	Leu	Glu	Asp	Glu	Ala	Ala	Gly	His	Val	
	50					55				60						
Thr	Gln	Ala	Arg	Met	Val	Ser	Lys	Ser	Lys	Asp	Gly	Thr	Gly	Ser	Asp	
	65			70					75					80		
Asp	Lys	Lys	Ala	Lys	Gly	Ala	Asp	Gly	Lys	Thr	Lys	Ile	Ala	Thr	Pro	
			85					90					95			
Arg	Gly	Ala	Ala	Pro	Pro	Gly	Gln	Lys	Gly	Gln	Ala	Asn	Ala	Thr	Arg	
		100						105					110			
Ile	Pro	Ala	Lys	Thr	Pro	Pro	Ala	Pro	Lys	Thr	Pro	Pro	Ser	Ser	Gly	
	115						120					125				
Glu	Pro	Pro	Lys	Ser	Gly	Asp	Arg	Ser	Gly	Tyr	Ser	Ser	Pro	Gly	Ser	
	130					135					140					
Pro	Gly	Thr	Pro	Gly	Ser	Arg	Ser	Arg	Thr	Pro	Ser	Leu	Pro	Thr	Pro	
	145				150				155						160	
Pro	Thr	Arg	Glu	Pro	Lys	Lys	Val	Ala	Val	Val	Arg	Thr	Pro	Pro	Lys	
		165					170							175		
Ser	Pro	Ser	Ser	Ala	Lys	Ser	Arg	Leu	Gln	Thr	Ala	Pro	Val	Pro	Met	
		180					185						190			
Pro	Asp	Leu	Lys	Asn	Val	Lys	Ser	Lys	Ile	Gly	Ser	Thr	Glu	Asn	Leu	

-continued

195						200					205				
Lys	His	Gln	Pro	Gly	Gly	Gly	Lys	Val	Gln	Ile	Val	Tyr	Lys	Pro	Val
	210					215					220				
Asp	Leu	Ser	Lys	Val	Thr	Ser	Lys	Cys	Gly	Ser	Leu	Gly	Asn	Ile	His
225					230					235					240
His	Lys	Pro	Gly	Gly	Gly	Gln	Val	Glu	Val	Lys	Ser	Glu	Lys	Leu	Asp
				245					250					255	
Phe	Lys	Asp	Arg	Val	Gln	Ser	Lys	Ile	Gly	Ser	Leu	Asp	Asn	Ile	Thr
			260					265					270		
His	Val	Pro	Gly	Gly	Gly	Asn	Lys	Lys	Ile	Glu	Thr	His	Lys	Leu	Thr
		275					280					285			
Phe	Arg	Glu	Asn	Ala	Lys	Ala	Lys	Thr	Asp	His	Gly	Ala	Glu	Ile	Val
	290					295					300				
Tyr	Lys	Ser	Pro	Val	Val	Ser	Gly	Asp	Thr	Ser	Pro	Arg	His	Leu	Ser
305					310					315					320
Asn	Val	Ser	Ser	Thr	Gly	Ser	Ile	Asp	Met	Val	Asp	Ser	Pro	Gln	Leu
				325					330					335	
Ala	Thr	Leu	Ala	Asp	Glu	Val	Ser	Ala	Ser	Leu	Ala	Lys	Gln	Gly	
			340					345					350		

What is claimed is:

1. A method for confirming a clinical diagnosis of Alzheimer's Disease in a patient suspected of having Alzheimer's Disease, comprising performing an assay to determine the concentration of tau-peptide in the patient's blood, wherein the assay comprises contacting a sample of blood obtained from the patient with an antibody which specifically binds tau-peptide or a Fab fragment which specifically binds tau-peptide, wherein further the tau-peptide is derived from the amino terminal 200 amino acids or carboxy terminal 50 amino acids of a tau-protein; and determining the concentration of tau-peptide wherein an elevated concentration of

tau-peptide confirms the clinical diagnosis of Alzheimer's Disease.

2. The method of claim 1, wherein the assay comprises an assay selected from the group consisting of a radioimmunoassay, an enzyme-linked immunosorbant assay, a sandwich assay, a precipitin reaction, a gel immunodiffusion assay, an agglutination assay, a fluorescent immunoassay, a protein A immunoassay and an immunoelectrophoresis assay.

* * * * *

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PATENT AND TRADEMARK OFFICE
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IEX	1	5	4	\$1,014.00	4697059
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	DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE				
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ABSTRACT

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The present invention is directed to methods and kits for diagnosing, ~~subtyping and monitoring~~ Alzheimer's disease. The invention is based, in part,
5 on the discovery that proteolytic fragments of the amino and carboxy terminal amino acid residues of tau-proteins are released from the neurofibrillary tangles associated with the disease and can be detected in body fluids outside the brain. ←

10 The tau-proteins will be purified or chemically synthesized and peptide fragments of the amino terminal and carboxy terminal regions will be obtained proteolytically or synthesized chemically and will be used in generating tau specific antibodies for
15 use in diagnostic kits for the detection of Alzheimer's disease. These diagnostic kits will be used in screening the body fluids of individuals for the presence of tau-peptide fragments. Alternatively,
20 kits for screening the body fluids of individuals for the presence of circulating autoantibodies.

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1. FIELD OF THE INVENTION

The present invention is directed to the diagnosis, subtyping and monitoring of Alzheimer's disease. Diagnostic methods and kits are described which utilize monoclonal antibodies, polyclonal antibodies or Fab fragments specific to peptide fragments of the tau family of proteins which are found in the blood or spinal fluid of individuals affected by Alzheimer's disease. Alternatively, tau-peptides can be used to detect circulating autoantibodies in such patients.

The diagnostic Alzheimer's disease detection kits of the present invention can be particularly useful in screening the overall population and identifying those individuals who have Alzheimer's disease as well as subtyping the affected individuals according to which member of the tau family of proteins they express.

2. BACKGROUND OF THE INVENTION

Alzheimer's disease is the most common form of both senile and presenile dementia in the world and is recognized clinically as relentlessly progressive dementia that presents with increasing loss of memory, intellectual function and disturbances in speech (Merritt, 1979, *A Textbook of Neurology*, 6th edition, pp. 484-489 Lea & Febiger, Philadelphia). The disease itself usually has a slow and insidious progress that affects both sexes equally, worldwide. It begins with mildly inappropriate behavior, uncritical statements, irritability, a tendency towards grandiosity, euphoria and deteriorating performance at work; it progresses through deterioration in operational judgement, loss of insight, depression and loss of recent memory; it ends in severe disorientation and confusion, apraxia of gait, generalized rigidity and incontinence (Gilroy

& Meyer, 1979, *Medical Neurology*, pp. 175-179
MacMillan Publishing Co.). Alzheimer's disease
afflicts an estimated 4 million human beings in the
United States alone at a cost of 35 billion dollars a
5 year (Hay & Ernst, 1987, *Am. J. Public Health*,
77:1169-1175). It is found in 10% of the population
over the age of 65 and 47% of the population over the
age of 85 (Evans et al., 1989, *JAMA*, 262:2551-2556).
In addition, the disease is found at much lower levels
10 in the younger age groups, usually beginning at about
30 years of age and even rarely in late childhood
(Adams & Victor, 1977, *Principles of Neurology*, pp.
401-407).

The etiology of Alzheimer's disease is
15 unknown. Evidence for a genetic contribution comes
from several important observations such as the
familial incidence, pedigree analysis, monozygotic and
dizygotic twin studies and the association of the
disease with Down's syndrome (for review see
20 Baraitser, 1990, *The Genetics of Neurological*
Disorders, 2nd edition, pp. 85-88). Nevertheless,
this evidence is far from definitive and it is clear
that one or more other factors are also required.
Elevated concentrations of aluminum have been found in
25 the brains of some patients dying with Alzheimer's
disease (Crapper et al., 1976, *Brain*, 99:67-80) and
one case report has documented markedly elevated
levels of manganese in the tissues of a patient with
Alzheimer's disease (Banta & Markesberg, 1977,
30 *Neurology*, 27:213-216), which has led to the
suggestion that high levels of these metals may be
neurotoxic and lead to the development of Alzheimer's
disease. It was interesting that the aluminum ions
35 were found to be associated mainly with the nuclear
chromatin in brain regions most likely to display
neurofibrillary tangles in Alzheimer's disease.
However, from a statistical point of view the absolute

differences found for the aluminum levels between normal and Alzheimer brains were far from convincing. It has recently been suggested that defects in the transcriptional splicing of mRNA coding for the tau complex of microtubule associated proteins occur (for review see Kosik, 1990, *Curr. Opinion Cell Biol.*, 2:101-104) and/or that inappropriate phosphorylation of these proteins exists (Grundke-Igbak et al., 1986, *Proc. Natl. Acad. Sci. USA*, 83:4913-4917; Wolozin & Davies, 1987, *Ann. Neurol.* 22:521-526; Hyman et al., 1988, *Ann. Neurol.*, 23:371-379; Bancher et al., 1989, *Brain Res.*, 477:90-99). Furthermore, reduction in the enzymes involved in the synthesis of acetylcholine has led to the view of Alzheimer's disease as a cholinergic system failure (Danes & Moloney, 1976, *Lancet*, ii:1403-14). However, even if cholinergic neurons are most at risk in Alzheimer's disease, it appears likely that these reductions in enzyme activity are secondary to the degenerative process itself rather than causally related.

2.1 THE DIAGNOSIS OF ALZHEIMER'S DISEASE

The diagnosis of Alzheimer's disease at autopsy is somewhat time-consuming but is definitive. The gross pathological changes are found in the brain, which is underweight, and shows generalized atrophy of both the gray and white matter of the cerebral cortex, particularly in the temporal and frontal lobes, with widening of the sulci and narrowing of the gyri as well as compensatory symmetrical dilation of the third and lateral ventricles (Adams & Victor, 1977, *Principles of Neurology*, pp. 401-407; Merritt, 1979, *A Textbook of Neurology*, 6th edition, Lea & Febiger, Philadelphia, pp. 484-489). The histological changes in the brain are definitive. The most common lesion is the neurofibrillary tangle (Kidd, 1963, *Nature*, 197:192-193; Kidd, 1964, *Brain* 87:307-320), which

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consists of a tangled mass of paired helical filaments and occasional straight filaments located in the cytoplasm of affected neurons (Oyanagei, 1979, *Adv. Neurol. Sci.*, 18:77-88 (in Japanese) as quoted by Grundke-Iqbal et al., 1985, *Acta Neuropathol.*, 66:52-61). The other characteristic histological lesions are Hirano bodies within nerve cells of certain brain regions, neuritic dystrophy and amyloid angiopathy. In addition, there are prominent argentophilic senile neuritic plaques composed of enlarged, degenerating axonal endings, containing degraded cellular organelles and some paired helical filaments, that surround a core of mainly extracellular amyloid (Merritt, 1979, *A Textbook of Neurology*, 6th edition, Lea & Febiger, Philadelphia, pp. 484-489). Small amounts of these histological changes are found also in the brains of almost all very old humans at autopsy, even if they showed no symptoms of Alzheimer's disease before death (Tomlinson & Henderson, 1976, *Neurobiology of Aging*, p. 183, Terry & Gershon (eds.), Raven, New York; Ball, 1976, *Neuropathol. Appl. Neurobiol.*, 2:395; Ball, 1977, *Acta Neuropathol.*, 37:111-118). However, the number and total amount of these histological changes in brain has been found to correlate well with the severity of the clinical signs and symptoms of Alzheimer's disease (Kidd, 1963, *Nature*, 197:192-193; Terry et al., 1964, *Am. J. Pathol.*, 44:269-297; Roth et al., 1966, *Nature*, 209:109-110; Blessed et al., 1968, *Br. J. Psychiat.*, 114:797-881; Tomlinson et al., 1970, *J. Neurol. Sci.*, 11:205-242; Wisniewski, 1976, *J. Neurol. Sci.*, 27:173-181).

The diagnosis of Alzheimer's disease during life is more difficult than at autopsy since it depends upon an inexact clinical evaluation. In the middle stages and particularly in the earlier stages of the disease, the diagnosis is a matter of clinical

judgement and success is markedly dependent upon the experience and clinical acumen of the attending physician or consultant. In the late stages of the disease however, clinical diagnosis is relatively
5 straight-forward even if it is not completely unequivocal. In all cases, a wide variety of other diseases, presenting at least partially overlapping clinical pictures, must be positively ruled out before a diagnosis of Alzheimer's disease can be made.
10 Usually a patient must be evaluated on a number of occasions over some period of time in order to document the deterioration in intellectual ability and other signs and symptoms before a diagnosis can be attempted with reasonable confidence. The necessity
15 of repeated evaluation by a professional medical practitioner before a firm diagnosis can be made is costly, generates anxiety and can be frustrating to patients and their families. Furthermore, the development of an appropriate therapeutic strategy is
20 greatly hampered by these difficulties in the objective and rapid diagnosis of the disease, particularly in the early stages where even a simple arrest of the progress of the disease would leave the patient with significant intellectual capacity and a
25 reasonable quality of life.

No unequivocal laboratory test specific for Alzheimer's disease has been reported. European patent application No. 391714 discloses a method for
30 the diagnosis of Alzheimer's disease based upon the detection of mature β -amyloid protein, its precursor protein or a fragment of β -amyloid protein in non-neural tissue biopsy using immunoassay techniques. However, this approach is non-specific since it has
35 been recognized that a wide variety of degenerative diseases, lead to the production and deposition of β -amyloid protein in almost all human tissues. Furthermore, even though the gene coding for both the

β -amyloid protein (Goldgaber et al., 1987, *Science*, 235:877-80; Tanzi et al., 1987a, *Science*, 235:880-884) and the gene for familial Alzheimer's disease (St. George-Hyslop et al., 1987, *Science*, 235:885-890) have
5 been located on human chromosome 21, it has been shown that the linkage between the two genes is not very close (Van Broedkhoven et al., 1987, *Nature*, 329:153-155; Tanzi et al., 1987b, *Nature*, 329:156-157). In addition, it is known that despite some earlier
10 claims, β -amyloid protein forms no part of the paired helical filaments within neurofibrillary tangles (for review see Wischik et al., 1989, *Curr. Opin. Cell Biol.*, 1:115-122). Therefore, even though β -amyloid protein is deposited in many tissues of patients with
15 Alzheimer's disease, it has not been definitively linked to Alzheimer's disease in a manner different to its association with many other degenerative diseases. Thus, the diagnostic usefulness of antibodies directed to mature β -amyloid protein, its precursor or any of
20 its fragments must be considered marginal at best.

2.2 NEUROFIBRILLARY TANGLES AND ALZHEIMER'S DISEASE

It is now known that the only definitive
25 feature of Alzheimer's disease is the presence of grossly increased quantities of neurofibrillary tangles within the affected cortical regions of brain compared to either normal brain from humans of any age or to brain from any disease state other than
30 Alzheimer's disease itself. However, the neurofibrillary tangles have not heretofore been exploited for use in a diagnostic or screening assay for Alzheimer's disease.

The progression of Alzheimer's disease is
35 characterized by a loss of cortical substance in the brain. This fact has been well documented over many years by many different researchers. The most

characteristic lesion in Alzheimer's disease is the presence of paired helical filaments in discrete, randomly interwoven groups or neurofibrillary tangles within affected cortical neurons. In addition, the
5 number and size of these tangles within an affected neuron as well as the total number of tangles and the total amount of constituent tau-protein in an affected brain correlates with the progression and severity of the disease. Therefore, it is believed that as these
10 tangles increase in size and number they must interfere with the physiological function of each cell in which they occur, eventually leading directly to the death and lysis of that cell. Recently, it has been reported that the cytosolic ATP-dependent
15 protease signal protein, ubiquitin, becomes attached to neurofibrillary tangles (Mori et al., 1987, *Science*, 235:1641-1644). Since ubiquitinated proteins are rapidly degraded, this suggests that the affected cell recognizes the tangles as a foreign structure
20 requiring degradation. It is further believed that this mechanism of cortical neuron death is responsible for the progressive loss of cortical substance and, therefore, the loss of intellectual capacity as well as the appearance of the other signs and symptoms of
25 Alzheimer's disease.

The paired helical filaments are composed of an unknown protein of 90,000 molecular weight (Mr) associated with various tau-protein species. A tightly bound helical core is formed between the
30 90,000 Mr protein and the large middle domain of the tau-protein as well as a region of the C-terminal domain, which contains the imperfect tandem repeat. The structural conformation of the helical core allows the N-terminal and C-terminal domains of the tau-
35 protein to protrude at some angle from the axis of the paired helical filament thereby forming a protease-sensitive coat around the paired helical filaments

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(Wischnik et al., 1988, *Proc. Natl. Acad. Sci. USA.*,
85:4884-4888). The research groups working in this
area are directing their efforts towards
characterizing the protease-resistant core of the
5 paired helical filaments in order to understand the
nature of the stable interaction and the nature of the
modification that occurs to tau-proteins in
Alzheimer's disease (for reviews, see Wischnik et al.,
1989, *Curr. Opin. Cell Biol.*, 1:115-122; Kosik,
10 1990, *Curr. Opin. Cell Biol.*, 2:101-104). This
orientation of research effort as well as research
directed towards the molecular genetics of the tau-
protein are likely to lead to an understanding of the
primary cause of Alzheimer's disease.

15 From the foregoing, it is clear that no one
concerned with Alzheimer's disease has realized that
its diagnosis ultimately must depend upon the
detection and quantitative measurement of the amount
of the component substance or substances that
20 constitute these neurofibrillary tangles in victims of
the disease. The detection of associated substances
that can also be found in other disease states can
never be as definitive as the detection of the actual
substance building up in affected neurons in
25 Alzheimer's disease causing the eventual death of
those cells. What has also not been realized is that
the protease stability of the core of the paired
helical filaments makes it unlikely that any part of
the tau-protein or the 90,000 Mr protein present in
30 this stable core will ever be found outside the brain.
The slow destruction of these stable cores, following
their release by dead neurons, can be conceived to
occur only *in situ* and as a consequence of
phagocytosis by microglia. The most likely reason for
35 the reluctance or failure to recognize the potential
of using components of the neurofibrillary tangles in
the diagnosis of Alzheimer's disease relates to the

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natural abhorrence that a person would feel if required to biopsy the brain of any living human being even in the last stages of the disease in order to measure these substances. The biopsy procedure is
5 extremely invasive and must leave the person so biopsied less than whole. However, the scientific community has not considered the possibility that biopsy might not be necessary in order to detect the substance or substances in question.

10 In contrast to the relevant literature, the diagnostic approach of the present invention is based on the recognition that the whole of the 200 amino acid N-terminal residues of the various tau-proteins as well as some portion of their 50 amino acid most C-
15 terminal residues will be released when cleaved from the filaments by ubiquitin-recognizing proteases or other proteases during degeneration and rupture of affected neurons. The diagnostic assays of the invention are based on the principle that it is these
20 cleaved latter segments of tau-proteins that can find their way into body fluids outside the brain and whose detection and quantitation are important for diagnostic purposes. Additional support for this last conclusion comes from the observation that other
25 brain-specific, soluble and protease-sensitive proteins find their way into blood and spinal fluid following head trauma (Phillips et al., 1980, *Br. Med. J.*, 281:777-779; Brayne et al., 1982, *Lancet*, ii:1308-9) and various other types of neurological disorders
30 (Thompson et al., 1980, *J. Neurol. Sci.*, 47:241-254; Willson et al., 1980, *Ann. Clin. Biochem.*, 17:110-113).

3. SUMMARY OF THE INVENTION

35 The present invention relates to methods and kits for diagnosing, subtyping and monitoring Alzheimer's disease. The invention is based, in part,

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on the discovery that proteolytic fragments of the amino and carboxy terminal amino acid residues of tau-proteins are released from the neurofibrillary tangles associated with the disease and can be detected in
5 body fluids outside the brain.

The generation and purification of tau-proteins and peptide fragments of the amino terminal and carboxy terminal regions of tau-proteins are described. These tau-proteins and fragments thereof
10 may be used to generate monoclonal antibodies, polyclonal antibodies or Fab fragments that, in turn, can be used in immunoassays to detect circulating tau-peptides in suspected individuals. Alternatively, the tau-peptides themselves may be used in immunoassays to
15 detect circulating autoantibodies in such individuals.

3.1 DEFINITIONS

The term tau-protein is defined herein as any one of the proteins that comprise a single
20 constituent part of the tau-complex of proteins.

The tau-complex of proteins is defined as that group of microtubule associated proteins (MAPS) from vertebrate brain that have the following characteristics: (a) co-purify with tubulin during the
25 cycle purification of microtubules, particularly in the absence of glycerol (Asnes & Wilson, 1979, *Anal. Biochem.*, **98**:64-73; Murphy & Hiebsch, 1979, *Anal. Biochem.*, **96**:225-235); (b) separate from tubulin present in the microtubules obtained by the cycle
30 technique when an ice-cold sample of such a preparation is chromatographed on a phosphocellulose column, using 2-(N-morpholino)-ethanesulphonic acid (MES) buffer to remove tubulin and using MES buffer containing 1 M NaCl to remove the tau-complex of
35 proteins (Weingarten *et al.*, 1975, *Proc. Natl. Acad. Sci. USA*, **72**:1858-1862; Cleveland *et al.*, 1977, *J. Mol. Biol.*, **116**:207-225; Cleveland *et al.*, 1979, *J.*

Biol. Chem., 254:12670-12678); (c) are included in a column of Sepharose 6B when chromatographed on such a matrix and where they migrate as an aggregated species not far from the exclusion volume but have individual
5 unaggregated native molecular masses between 10,000 and 100,000 Mr regardless of axial ratio (Cleveland et al., 1977, *J. Mol. Biol.*, 116:207-225); (d) react with any of a number of antibodies described in the literature as "anti-tau" or with a commercial antibody
10 described as anti-tau; (e) contain within each and every one of their C-terminal domains an imperfect tandem repeat of the type described in the literature (Lewis et al., 1988, *Science*, 242:936-939; Goedert et al., 1989, *EMBO J.*, 8:393-399; Kanai et al., 1989, *J.*
15 *Cell Biol.*, 109:1173-1184); and (f) are either heat-stable or heat-labile as described by Cleveland et al., 1977, *J. Mol. Biol.*, 116:207-225.

The term "forward sandwich assay" is defined in its most basic form as an assay wherein the sample
20 to be tested containing the tau-peptide reacts with an anti-tau-peptide antibody that has been immobilized upon a solid substrate; then a labeled second anti-tau-peptide antibody is added, which reacts with the tau-peptide at a different site.

25 The term "reverse sandwich assay" is defined in its most basic form as an assay wherein the labeled anti-tau-peptide antibody and sample to be tested containing the tau-peptide are first incubated together and then added to a second unlabeled anti-
30 tau-peptide antibody that had previously been immobilized upon a solid substrate as in the "forward sandwich assay". Both the labeled and the unlabeled anti-tau-peptide must react with the tau-peptide at a
35 different site.

✓ 4. DESCRIPTION OF THE FIGURE (SEQ ID NO: 1)

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Figure 1 shows the complete nucleotide and amino acid sequences (SEQ ID NO: 2) of one form of human tau-protein, reproduced from Goedert et al., 1988, Proc. Natl.

5 Acad. Sci. USA, 85:4051-4055.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention results from the unexpected discovery of several physiological functions for individual proteins in the tau-complex of proteins that have not heretofore been described and are in addition to the well recognized function of promoting the polymerization of microtubules and/or their stability. This discovery has permitted the development of assays for the individual proteins in the tau-complex of proteins and their subsequent purification with retention of their native configuration and physiological activity. The invention is based, in part, on the discovery that in patients suffering from Alzheimer's disease there is proteolytic fragmentation of the tau-complex of proteins which occurs independently of phosphorylation, such that only the N-terminal domain comprising up to not more than the 200 most N-terminal amino acids or less and the C-terminal domain comprising up to only the 50 most C-terminal amino acids or less of tau will be released from the paired helical filaments, resident in the neurofibrillary tangle or free in the cytoplasm of affected neurons, and make their way into spinal fluid and blood.

This invention provides the first demonstration that any part of the tau-proteins of brain in victims of Alzheimer's disease are also present in body fluids outside the brain itself in concentrations greatly exceeding that found in normal humans that are not victims of Alzheimer's disease. This is achieved by examining a sample of body fluid,

preferably blood or spinal fluid, and detecting the presence and quantity of any of the *tau*-proteins or fragments thereof. The detection and quantitative measurement of any of these *tau*-proteins or fragments thereof in body fluids in accordance with this invention is useful in confirming a clinical diagnosis of Alzheimer's disease in affected patients and in following the course of the disease. The invention is also useful in monitoring the disease during and subsequent to a period of treatment with agents that are being tested for their ability to stabilize, decrease or prevent the release of *tau*-proteins or fragments thereof from the brain.

Alternatively, the invention is directed to the production of *tau*-peptides for use in detecting circulating anti-*tau*-peptide autoantibodies in body fluids outside the brain in victims of Alzheimer's disease. The detection of circulating anti-*tau*-peptide autoantibodies in accordance with this invention is useful in confirming a clinical diagnosis of Alzheimers disease in affected patients and in following the course of the disease.

For purposes of description only, the invention will be described in terms of: (a) generating *tau*-peptides; (b) generating antibodies that define the *tau*-peptide; and (c) diagnostic assays and kits for diagnosing subtyping or monitoring Alzheimer's disease.

30 5.1 PRODUCTION OF TAU-PEPTIDES

5.1.1 ENZYMATIC CLEAVAGE OF PURIFIED TAU-PROTEINS

The production of *tau*-peptides may be accomplished by isolating the paired helical filaments from human brains of patients who died with Alzheimers disease and purifying the helical complex containing the *tau*-protein and the 90,000 Mr protein (see Section 6, *infra*). The *tau*-protein is associated with the

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90,000 Mr protein such that the 200 most amino terminal residues and the 50 most carboxy terminal residues protrude from the helical core of the complex thereby rendering those fragments susceptible to proteolytic enzymes.

The cleavage of the tau-protein into two large fragments of amino acid residues 1 to 151 and 154 to 352 and the dipeptide, Ser-Arg, may be accomplished using either one of the arginine-specific proteolytic enzymes such as thrombin (EC 3.4.13) as described recently (Joly et al., 1989, *J. Cell Biol.*, 109:2289-2294) or clostridiopeptidase B (clostripain) (EC 3.4.4.20). The preferred embodiment uses clostripain because the enzyme is a sulphydryl enzyme, requiring calcium ions for full activity. Consequently, its activity can be terminated conveniently by the addition of excess iodoacetamide and ethylene glycol-bis(β -aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA). In the case of either protease the concentration of tau-protein is adjusted to 100 μ g/ml before beginning the digestion. Purified clostripain is used at a concentration of 4 international units/ml in the following buffer (50 mM phosphate buffer pH 7.7, 1 mM β -mercaptoethanol, and 1 mM calcium acetate). Soybean trypsin inhibitor (clostripain/inhibitor molar ratio = 10/1) is added in order to eliminate any small residual trypsin activity in commercially available preparations of the enzyme. The cleavage reaction is conducted at 37°C and is terminated by the addition of a sufficient volume of a 100 mM stock solution EGTA, pH 7.7, containing 100 mM iodoacetimide to give a final concentration of 2 mM EGTA in the protease reaction mixture. The exact time required for proteolysis varies with each tau-protein and markedly depends upon the batch of clostripain used. Therefore, the optimum time for a single cleavage must be determined for each combination of

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clostripain batch and tau-protein used. The time course of the cleavage reaction is determined by conducting the reaction as described above, but on a 20 μ l volume and assaying the cleavage reaction by
5 subjecting samples at various times to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) according to the method of Laemmli, (*Nature*, 1970, 227:680-684) as modified by Studier, *Sci.*, 176:367-376 (1972) using a 10% (w/v) polyacrylamide resolving gel
10 followed by Serva blue staining and destaining in the usual manner of anyone familiar with the art.

 The two large tau-peptide fragments resulting from either thrombin or clostripain proteolysis may be further cleaved by digestion with
15 trypsin, which cleaves on the carboxy terminus of lysine or arginine residues. See for example, the techniques described in *Current Protocols in Molecular Biology*, Ausubel et al., Green Publish. Assoc. & Wiley Interscience, Ch. 10.

20 The sequence of tau-peptides derived from proteolytic digestion may be identified using the Edman degradation method of protein sequencing. This method sequentially removes one amino acid residue at a time from the amino terminal end of a peptide for
25 subsequent sequence identification by chromatographic procedures. See for example, the techniques described in Konigsberg and Steinman, 1977, *Strategy and Methods of Sequence Analysis*, in Neurath and Hill (eds.), *The Proteins* (3rd ed.) Vol. 3, pp. 1-178, Academic Press.
30 In addition, sequence analysis of tau-peptides may be accelerated by using an automated liquid phase amino acid sequenator following recently described techniques (Hewick et al., 1981, *J. Biol. Chem.*, 256:7990-7997; Stein and Undefriend, 1984, *Analy. Chem.*, 136:7-23), thereby allowing for the analysis of
35 picomolar quantities of tau-peptides containing up to 50 amino acid residues in length.

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5.1.2 CHEMICAL SYNTHESIS OF TAU-PEPTIDES

Tau-peptides can also be produced by chemical synthesis of the amino acid sequence of a tau-protein (Goedert et al., 1988, *Proc. Natl. Acad. Sci. USA*, **85**:4051-4055), as predicted from the cloning and sequencing of a cDNA coding for a tau-protein. This tau-protein sequence information may be utilized to predict the appropriate amino and carboxy terminal tau-peptides to be chemically synthesized using standard peptide synthesis methods known in the art. These methods include a solid-phase method devised by R. Bruce Merrifield, (Erickson and Merrifield, "Solid-Phase Peptide Synthesis", in *The Proteins*, Volume 2, H. Neurath & R. Hill (eds.) Academic Press, Inc., New York pp. 255-257; Merrifield, 1986, "Solid phase synthesis", *Science*, **242**:341-347). In the solid-phase method, amino acids are added stepwise to a growing peptide chain that is linked to an insoluble matrix, such as polystyrene beads. A major advantage of this method is that the desired product at each stage is bound to beads that can be rapidly filtered and washed and thus the need to purify intermediates is obviated. All of the reactions are carried out in a single vessel, which eliminates losses due to repeated transfers of products. This solid phase method of chemical peptide synthesis can readily be automated making it feasible to routinely synthesize peptides containing about 50 residues in good yield and purity (Stewart and Young, 1984, *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chemical Co.; Tam et al., 1983, *J. Am. Chem. Soc.*, **105**:6442). For example, tau-peptides corresponding to amino acid residues 1 to 30 and 331 to 352 as depicted in Figure 1 could be synthesized.

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**5.1.3 CLONING AND EXPRESSION OF RECOMBINANT
TAU-PROTEINS AND/OR TAU-PEPTIDES**

The production of tau-peptides can further be achieved by recombinant DNA technology. For example, appropriate tau nucleotide coding sequences
5 may be synthesized, cloned and expressed in appropriate host cells. Since the DNA sequence encoding for a tau-protein is known (Goeddert et al., 1988, *Proc. Natl. Acad. Sci., USA* **85**:4051-4055), DNA
10 probes may be synthesized by standard methods known in the art to screen cDNA libraries prepared from brain tissue of Alzheimer's disease patients for the specific tau-protein cDNA's. These DNA probes can further be used to isolate the entire family of tau-
15 protein genes from these cDNA libraries using methods which are well known to those skilled in the art. See, for example, the techniques described in Maniatis et al., 1982, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y., Chapter 7.

20 The polymerase chain reaction (PCR) technique can be utilized to amplify the individual members of the tau family for subsequent cloning and expression of tau-protein cDNAs (e.g., see U.S. patent numbers 4,683,202; 4,683,195; 4,889,818; Gyllensten et
25 al., 1988, *Proc. Nat'l Acad. Sci. USA*, **85**:7652-7656; Ochman et al., 1988, *Genetics*, **120**:621-623; Triglia et al., 1988, *Nucl. Acids. Res.*, **16**:8156; Frohman et al., 1988, *Proc. Nat'l Acad. Sci. USA*, **85**:8998-9002; Loh et al., 1989, *Science*, **243**:217-220).

30 Methods which are well known to those skilled in the art can be used to construct expression vectors containing tau-proteins or fragments thereof coding sequences and appropriate transcriptional/translational control signals. These
35 methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques

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described in Maniatis et al., 1982, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y., Chapter 12.

A variety of host-expression vector systems
5 may be utilized to express tau-proteins or fragments thereof. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing a coding sequence
10 for a tau-protein or fragment thereof; yeast transformed with recombinant yeast expression vectors containing a coding sequence for a tau-protein or fragment thereof; insect cell systems infected with recombinant virus expression vectors (e.g.,
15 baculovirus) containing a coding sequence for a tau-protein or fragment thereof; or animal cell systems infected with recombinant virus expression vectors (e.g., adenovirus, vaccinia virus) containing a coding sequence for a tau-protein or fragment thereof.

20 The expression elements of these vectors vary in their strength and specificities. Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be
25 used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when
30 cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when cloning in mammalian cell systems, promoters such as the adenovirus late promoter or the vaccinia virus 7.5K promoter may be used. Promoters produced by
35 recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted coding sequence for a tau-protein or fragment thereof.

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In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience Ch. 13; Grant et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 1987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C. Ch.3; and Bitter, 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast *Saccharomyces*, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II. For complementation assays in yeast, cDNAs for tau-proteins or fragments thereof may be cloned into yeast episomal plasmids (YEp) which replicate autonomously in yeast due to the presence of the yeast 2μ circle. The tau-protein or fragment thereof sequence may be cloned behind either a constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL (Cloning in Yeast, Chpt. 3, R. Rothstein In; DNA Cloning Vol. 11, A Practical Approach, Ed. DM Glover, 1986, IRL Press, Wash., D.C.). Constructs may contain the 5' and 3' non-translated regions of a cognate tau-protein mRNA or those corresponding to a yeast gene. YEp plasmids transform at high efficiency and the plasmids are extremely stable. Alternatively vectors may be used which promote integration of foreign DNA sequences into the yeast chromosome.

A particularly good expression system which could be used to express tau-proteins or fragments thereof is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The

tau-protein or fragment thereof coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the polyhedrin gene results in production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (e.g., see Smith et al., 1983, *J. Biol.*, **46**:586; Smith, U.S. Patent No. 4,215,051).

In cases where an adenovirus is used as an expression vector, the tau-protein or fragment thereof coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vivo* or *in vitro* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the tau-protein or fragment thereof in infected hosts. (e.g., See Logan & Shenk, 1984, *Proc. Natl. Acad. Sci.*, (USA) **81**:3655-3659). Alternatively, the vaccinia 7.5K promoter may be used. (e.g., see Mackett et al., 1982, *Proc. Natl. Acad. Sci.*, (USA) **79**:7415-7419; Mackett et al., 1984, *J. Virol.*, **49**:857-864; Panicali et al., 1982, *Proc. Natl. Acad. Sci.*, **79**:4927-4931).

Specific initiation signals may also be required for efficient translation of the inserted tau-protein or fragment thereof coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where the entire tau-protein genome, including its own initiation codon and adjacent sequences, are inserted into the appropriate

expression vectors, no additional translational control signals may be needed. However, in cases where only a portion of the tau-protein coding sequence is inserted, exogenous translational control signals, including the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the tau-protein or fragment thereof coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bitter et al., 1987, *Methods in Enzymol.*, 153:516-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression driven by certain promoters can be elevated in the presence of certain inducers, (e.g., zinc and cadmium ions for metallothionein promoters). Therefore, expression of the genetically engineered tau-protein or fragment thereof may be controlled. This is important if the protein product of the cloned foreign gene is lethal to host cells. Furthermore, modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

The host cells which contain the tau-protein or fragment thereof coding sequence and which express the biologically active tau-protein or fragment

thereof gene product may be identified by at least four general approaches: (a) DNA-DNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by expression of tau-protein mRNA transcripts in host cells; and (d) detection of tau-protein gene products as measured by immunoassays or by its biological activity.

In the first approach, the presence of the tau-protein or fragment thereof coding sequence inserted in the expression vector can be detected by DNA-DNA hybridization using probes comprising nucleotide sequences that are homologous to the tau-protein coding sequence or particular portions thereof substantially as described recently (Goeddert et al., 1988, *Proc. Natl. Acad. Sci. USA*, **85**:4051-4055).

In the second approach, the recombinant expression vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the tau-protein or fragment thereof coding sequence is inserted within a marker gene sequence of the vector, recombinants containing the tau-protein or fragment thereof coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed in tandem with the tau-protein or fragment thereof coding sequence under the control of the same or different promoter used to control the expression of the tau coding sequence. Expression of the marker in response to induction or selection indicates expression of the tau-protein coding sequence.

In the third approach, transcriptional activity for the tau-protein or fragment thereof

coding region can be assessed by hybridization assays. For example, RNA can be isolated and analyzed by Northern blot using a probe homologous to the tau-protein or fragment thereof coding sequence or
5 particular portions thereof substantially as described (Goeddert et al., 1988, *Proc. Natl. Acad. Sci. USA*, 85:4051-4055). Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

10 In the fourth approach, the expression of the tau-protein or fragment thereof product can be assessed immunologically, for example by Western blots, immunoassays such as radioimmunoprecipitation, enzyme-linked immunoassays and the like.

15 Once a recombinant that expresses a tau-protein or fragment thereof is identified, the gene product should be analyzed. This can be achieved by assays based on the physical, immunological or functional properties of the product.

20 A tau-protein or fragment thereof should be immunoreactive whether it results from the expression of the entire gene sequence, a portion of the gene sequence or from two or more gene sequences which are ligated to direct the production of chimeric proteins.
25 This reactivity may be demonstrated by standard immunological techniques, such as radioimmunoprecipitation, radioimmune competition, or immunoblots.

30 **5.2 GENERATION OF ANTIBODIES THAT
DEFINE TAU-PEPTIDES**

Various procedures known in the art may be used for the production of antibodies to epitopes of the tau-protein. Such antibodies include but are not
35 limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and an Fab expression library. For the production of antibodies, various host animals

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may be immunized by injection with a particular tau-protein, or a synthetic tau-peptide, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *corynebacterium parvum*.

Monoclonal antibodies to peptides of tau may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, (*Nature*, 1975, 256:495-497), the more recent human B-cell hybridoma technique (Kosbor et al., 1983, *Immunology Today*, 4:72) and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention monoclonal antibodies specific to tau-peptides may be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, *Proc. Natl. Acad. Sci.*, 80:2026-2030) or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, in, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.*, 81:6851-6855; Neuberger et al., 1984, *Nature*, 312:604-608; Takeda et al., 1985, *Nature*, 314:452-454) by splicing the genes

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from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce tau-peptide-specific single chain antibodies.

An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity to tau-peptides.

Antibody fragments which contain specific binding sites of tau-peptides may be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments.

5.3 DIAGNOSTIC ASSAYS AND KITS FOR ALZHEIMER'S DISEASE

Yet another purpose of the present invention is to provide reagents for use in diagnostic assays for the detection of tau-peptides from individuals suffering from Alzheimer's disease.

In one mode of this embodiment, tau-peptides of the present invention may be used as antigens in immunoassays for the detection of those individuals suffering from Alzheimer's disease. The proteins, polypeptides and peptides of the present invention may be used in any immunoassay system known in the art including, but not limited to: radioimmunoassays,

enzyme-linked immunosorbent assay, "sandwich" assays, precipitin reactions, gel diffusion immunodiffusion assays, agglutination assays, fluorescent immunoassays, protein A immunoassays and
5 immuno-electrophoresis assays, to name but a few. U.S. Patent No. 4,629,783 and patents cited therein also describe suitable assays.

According to the present invention, monoclonal or polyclonal antibodies produced to
10 various forms of the tau-protein, can be used in an immunoassay on samples of blood, spinal fluid or other body fluid to diagnose patients with Alzheimer's disease.

In practicing the invention, a sample of
15 blood is removed from the patient by venesection and placed in contact with an anticoagulant such as EDTA, mixed, centrifuged at 600 g for 10 min and the plasma removed as is common in the art or a sample of spinal fluid is removed from the patient by lumbar puncture.

20 The antibodies described in Section 5.2 supra may be used as the basic reagents in a number of different immunoassays to determine the presence of a particular tau-peptide in a sample of blood or spinal fluid. Generally speaking, the antibodies can be
25 employed in any type of immunoassay, whether qualitative or quantitative. This includes both the two-site sandwich assay and the single site immunoassay of the non-competitive type, as well as in traditional competitive binding assays.

30 Particularly preferred, for ease of detection, and its quantitative nature, is the sandwich or double antibody assay, of which a number of variations exist, all of which are intended to be encompassed by the present invention.

35 For example, in a typical forward sandwich assay, unlabeled antibody is immobilized on a solid substrate, e.g., microtiter plate wells, and the

sample to be tested is brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen binary complex, a
5 second antibody, labelled with a reporter molecule capable of inducing a detectable signal, is then added and incubation is continued allowing sufficient time for binding with the antigen at a different site and the formation of a ternary complex of antibody-
10 antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal, which may be quantitated by comparison with a control sample containing known amounts of antigen. Variations on
15 the forward sandwich assay include the simultaneous assay, in which both sample and antibody are added simultaneously to the bound antibody, or a reverse sandwich assay in which the labelled antibody and sample to be tested are first combined, incubated and
20 added to the unlabelled surface bound antibody. These techniques are well known to those skilled in the art, and the possibility of minor variations will be readily apparent. As used herein, "sandwich assay" is intended to encompass all variations on the basic two-
25 site technique.

For the sandwich assays of the present invention, the only limiting factor is that both antibodies have different binding specificities for the tau-peptide. Thus, a number of possible
30 combinations are possible.

As a more specific example, in a typical forward sandwich assay, a primary antibody is either covalently or passively bound to a solid support. The solid surface is usually glass or a polymer, the most
35 commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinylchloride or polypropylene. The solid supports may be in the

form of tubes, beads, discs or microplates, or any other surfaces suitable for conducting an immunoassay. The binding processes are well known in the art. Following binding, the solid phase-antibody complex is washed in preparation for the test sample. An aliquot of the body fluid containing the tau-peptide to be tested is then added to the solid phase complex and incubated at 25°C for a period of time sufficient to allow binding of any tau-peptide present to the antibody specific for tau-peptide. The second antibody is then added to the solid phase complex and incubated at 25°C for an additional period of time sufficient to allow the second antibody to bind to the primary antibody-antigen solid phase complex. The second antibody is linked to a reporter molecule, the visible signal of which is used to indicate the binding of the second antibody to any antigen in the sample. By "reporter molecule", as used in the present specification is meant a molecule which by its chemical nature, provides an analytically detectable signal which allows the detection of antigen-bound antibody. Detection must be at least relatively quantifiable, to allow determination of the amount of antigen in the sample, this may be calculated in absolute terms, or may be done in comparison with a standard (or series of standards) containing a known normal level of antigen.

The most commonly used reporter molecules in this type of assay are either enzymes or fluorophores. In the case of an enzyme immunoassay an enzyme is conjugated to the second antibody, often by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are well known to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase and alkaline phosphatase, among others.

The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine or toluidine are commonly used. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-tau-peptide complex and allowed to bind to the complex, then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the tertiary complex of antibody-antigen-labelled antibody. The substrate reacts with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an evaluation of the amount of antigen which is present in the serum sample.

Alternately, fluorescent compounds, such as fluorescein or rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic longer wavelength. The emission appears as a characteristic color visually detectable with a light microscope. As in the enzyme immunoassay (EIA), the fluorescent-labelled antibody is allowed to bind to the first antibody-tau-peptide complex. After washing the unbound reagent, the remaining ternary complex is then exposed to light of the appropriate wavelength, and the fluorescence observed indicates the presence of the antigen.

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Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent or bioluminescent molecules may also be employed. It will be readily apparent to the skilled artisan how to vary the procedure to suit the required use.

Alternatively, the sample to be tested either human blood or spinal fluid containing the tau-peptide may be used in a single site immunoassay wherein it is adhered to a solid substrate either covalently or noncovalently. An unlabeled anti-tau-peptide antibody is brought into contact with the sample bound on the solid substrate. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen binary complex a second antibody, labelled with a reporter molecule capable of inducing a detectable signal, is then added and incubation is continued allowing sufficient time for the formation of a ternary complex of antigen-antibody-labeled antibody. For the single site immunassay, the second antibody may be a general antibody (i.e., xenogeneic antibody to immunoglobulin, particularly anti-(IgM and IgG) linked to a reporter molecule) that is capable of binding an antibody that is specific for the tau-peptide of interest.

Alternatively, the purified tau-proteins or fragments thereof, described in Section 5.1, *supra*, may be used in a diagnostic assay to detect circulating anti-tau-peptide autoantibodies wherein they are adhered to a solid support either covalently or non-covalently. The sample, either human blood or spinal fluid diluted in an appropriately buffered medium, is added to the solid support and a sufficient time is allowed for complex formation between the tau-proteins or fragments thereof and any cognate

autoantibodies in the sample. The supernatant is removed and the solid support is washed to remove any non-specifically bound proteins. To the complex may be added xenogeneic antisera to human immunoglobulin, particularly anti-(human Ig M and Ig G) in an appropriately buffered medium. The xenogeneic antisera will normally be labeled with a reporter molecule as described above for the forward sandwich assay. Instead of xenogeneic antisera, proteins specific for the immune complex may be employed, e.g., *S. aureus* protein A. The label may then be detected as described above for the forward sandwich assay.

6. EXAMPLE: PRODUCTION AND PURIFICATION OF IMMUNOGENS FOR ANTIBODY PRODUCTION

6.1. PURIFICATION OF MICROTUBULES FROM BOVINE BRAIN BY THE CYCLE PROCEDURE AS A SOURCE OF NATIVE, PHYSIOLOGICALLY ACTIVE MICROTUBULE ASSOCIATED PROTEINS

The cycle purification of microtubules from bovine brain was performed in the presence of guanosine triphosphate and low concentrations of magnesium ions in order to obtain high ratios of microtubule associated proteins to tubulin. This cycle-purification was carried out in five separate steps according to a modification of the method described by Asnes & Wilson, (1979, *Anal. Biochem.*, 98:64-73).

Step (1) Transport & Dissection: Eight bovine brains (approximately 350 gram (g) each) were removed, cut into pieces, placed in ice-cold transport buffer [20 mM phosphoric acid (adjusted to pH 6.75 with 2M NaOH) 100 mM sodium glutamate, 300 mM sucrose] containing ice cubes made from transport buffer and transported in an ice bucket from the abattoir to the laboratory. The meninges, superficial blood vessels and the majority of the white matter was removed and

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discarded. The remaining brain tissue (approximately 1200 g) was drained and weighed.

Step (2) Homogenization: The selected brain tissue was dispersed using two separate maneuvers sequentially. The weighed brain tissue from step (1) was passed once through a domestic electric mincer and added to homogenization buffer [200 mM phosphoric acid (adjusted to pH 6.75 with 2 M NaOH), 100 mM sodium glutamate, 300 mM sucrose, 4 mM β -mercaptoethanol, 0.3 mM phenylmethanesulphonylfluoride (PMSF) (stock solution, 100 mM in dimethylformamide (DMF)), 0.3 mM N α -p-tosyl-1-lysine chloromethylketone (TLCK) (prepared in PMSF stock solution), 50 μ g/ml leupeptin, few crystals of catalase] (at 0°C and at a concentration of 1.5 ml buffer / g tissue). The material was dispersed by gentle stirring with a glass rod. The resulting suspension was then homogenized in three batches with two complete strokes (up and down twice for each batch separately) of a motor driven 1 liter (L) glass (2.2 inch diameter x 18 inch long) Potter - Elvehjem homogenizer fitted with a 2 inch long teflon pestle (0.005 inch clearance) at 0°C.

Step (3) First Cycle: Approximately 3000 ml of the homogenate was added to twelve 250 ml plastic centrifuge tubes and centrifuged at 27,880 g (13,000 rpm in two Sorval RC5B preparative centrifuges containing two GSA rotors precooled to 3°C) for 53 minutes at 0-4°C. The supernatants were carefully removed, combined and cofactor buffer [2.5 mM GTP, .50 mM MgCl₂, 1.0 mM EGTA (10 x stock solution adjusted to pH 6.75 at 0°C with NaOH)] prepared in homogenization buffer was added. The solution was mixed and then approximately 1L portions together with a glass thermometer was placed in separate polyethene sleeves (10 inch x 14 inch) and heat sealed. The loaded sleeves were gently agitated in a 50°C water bath until the temperature of their contents reached 30°C

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whereupon they were immediately transferred to a 30°C water bath where the incubation continued without further agitation for the remainder of a total period of 30 minutes. Assembly was followed viscometrically
5 using calibrated 100-size Cannon Ubbelohde glass viscometers. Approximately 2400 ml of the assembled extract was transferred to 12 clean 250 ml plastic centrifuge jars and centrifuged at 27,880 g (13,000 rpm in two Sorval RC5B preparative centrifuges
10 containing two Sorval GSA rotors prewarmed to 30°C) for 42 minutes at 30°C. The supernatant was carefully removed with an aspirator and its volume was measured, recorded and discarded.

Step (4) Second Cycle: The final pellet from
15 the first cycle was resuspended in ice-cold homogenization buffer without sucrose (10% of volume of the supernatant from the first cycle) using a precooled small Dounce homogenizer. Approximately 220 ml of the resuspended pellet was incubated at 0°C
20 for 30 minutes, added to six 36 ml plastic centrifuge tubes, and centrifuged at 39,000 g (14,627 rpm in a Sorval OTD centrifuge containing a Sorval AH-627 rotor precooled to 3°C) for 40 minutes at 0 to 4°C. The supernatant was carefully transferred to a 1L glass
25 conical flask and cofactor buffer was added. The solution was mixed and gently swirled in the 50°C water bath until the temperature reached 30°C. The flask was then immediately transferred to a 30°C water bath where the incubation continued without further
30 agitation for a total period of only 10 minutes. The extract was transferred to six 36 ml plastic centrifuge tubes and centrifuged at 39,000 g (14,726 rpm in a Sorval OTD ultracentrifuge containing a Sorval AH-627 rotor) for 30 minutes at 30°C. The
35 supernatant was removed carefully with an aspirator and its volume was measured and discarded.

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Step (5) Third Cycle - The final pellet from the second cycle was resuspended in ice-cold homogenization buffer without sucrose (40% of the volume of the supernatant from the second cycle) using a clean precooled small Dounce homogenizer. Approximately 90 ml of resuspended pellet was incubated at 0°C for 30 minutes, transferred to three 36 ml tubes, and centrifuged at 39,000 g (14,726 rpm in a Sorval OTD ultracentrifuge containing a Sorval AH-627 rotor with 36 ml buckets) for 40 minutes at 0 to 4°C. Approximately 80 ml of supernatant was carefully transferred to a 500 ml glass conical flask and cofactor buffer was added. The solution was mixed and gently swirled in the 50°C water bath until the temperature of the contents of the flask reached 30°C. Then the flask was immediately transferred to the 30°C water bath where the incubation continued without further agitation for a total period of 20 minutes. The warm extract was transferred to six 17 ml plastic centrifuge tubes and centrifuged at 39,000 g (Sorval PTD centrifuge containing a Sorval AH-627 rotor with 17 ml buckets) for 40 minutes at 30°C. The supernatant was removed carefully with an aspirator and discarded. Each tube contained a pellet of approximately 140 mg of microtubule (MT) proteins (approximately 50% tubulin and 50% MAPS) and was stored at -70°C.

30 **6.2. ISOLATION OF THE TAU CLASS OF
MICROTUBULE ASSOCIATED PROTEINS
FROM CYCLE PURIFIED MICROTUBULES**

The total MAPS fraction of cycle purified microtubules was separated from tubulin by a modification of the method of Weingarten, (1975, Proc. Natl. Acad. Sci. USA, 72:1858-1862). A pellet of microtubules from the cycle purification method was resuspended using a small Dounce homogenizer in MES

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buffer (0.1 M MES; 1 mM EGTA; 0.5 mM MgCl₂ and 1 mM β -mercaptoethanol) so that the resulting solution had an absorbance at 280 nm of 2.0 - 2.5 and then depolymerized by incubation for 30 min at 0°C. The
5 resulting solution was centrifuged at 100,000 g for 1 hour and the pellet discarded. The supernatant was filtered through a 0.2 μ milipore filter and applied to a phosphocellulose column (1.1 cm diameter x 2.6 cm length, 2.5 ml bed volume) that had previously been
10 equilibrated in MES buffer. Tubulin was eluted with MES buffer in the void volume of the column and when the absorbance of the eluate had returned to the baseline value, the total MAPS fraction was eluted (either 0.1 or 0.2 ml fractions) with MES buffer
15 containing 1.0 M NaCl and collected as a single complex peak of UV-absorbing material that contained multiple proteins.

The total MAPS fraction eluted from the phosphocellulose column was immediately desalted using
20 the rapid Sephadex G-25 centrifugation method of Penefsky (*J. Biol. Chem.*, 1977, **252**:2891-2899). The desalted total MAPS fraction was stirred at 0° C and solid ammonium sulphate added until its concentration reached 35% (w/v). The stirring was continued for 30
25 minutes and then the suspension was centrifuged at 20,000 g for 15 minutes. The supernatant was removed and again stirred at 0° C and more solid ammonium sulphate added until its concentration reached 45% (w/v). After a further 15 minute stirring the
30 suspension was again centrifuged at 20,000 g for 15 minutes and the supernatant discarded. The pellet from this last centrifugation step was dissolved in Mes buffer and applied to a column of hydroxyapatite (1 cm dia x 10 cm length) and eluted with Mes buffer.
35 The fractions containing only four closely spaced peptides of apparent Mr 45,000 to 65,000 by SDS-PAGE were combined. This total eluted tau fraction was

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reduced to a volume of 1.0 ml with a sartorius
collodion membrane operated in water under reduced
pressure, according to the instructions of the
manufacturer, and then divided into 0.1 ml portions in
5 0.5 ml plastic minifuge tubes and stored at -20°C.

The invention is not limited to the
embodiments described herein and may be modified or
varied without departing from the scope of the
invention.

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-37-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Voorheis, Paul H.
- (ii) TITLE OF INVENTION: Diagnostic Method for Alzheimer's Disease
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Pennie & Edmonds
 - (B) STREET: 1155 Avenue of the Americas
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 10036-2711
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: US 07/738,778
 - (B) FILING DATE: 01-AUG-1991
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Misrock, S. Leslie
 - (B) REGISTRATION NUMBER: 18,872
 - (C) REFERENCE/DOCKET NUMBER: 4697-040
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1107 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURE:
- (A) NAME/KEY: CDS
 - (B) LOCATION: 38..1092

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGCCTCTGT CGACTATCAG GTGAACCTTG AACCCAGG ATG GCT GAG CCC CGC CAG 55
Met Ala Glu Pro Arg Gln
1 5

GAG TTC GAA GTG ATG GAA GAT CAC GCT GGG ACG TAC GGG TTG GGG GAC 103
Glu Phe Glu Val Met Glu Asp His Ala Gly Thr Tyr Gly Leu Gly Asp
10 15 20

AGG AAA GAT CAG GGG GGC TAC ACC ATG CAC CAA GAC CAA GAG GGT GAC	151
Arg Lys Asp Gln Gly Gly Tyr Thr Met His Gln Asp Gln Glu Gly Asp	
25 30 35	
ACG GAC GCT GGC CTG AAA GCT GAA GAA GCA GGC ATT GGA GAC ACC CCC	199
Thr Asp Ala Gly Leu Lys Ala Glu Glu Ala Gly Ile Gly Asp Thr Pro	
40 45 50	
AGC CTG GAA GAC GAA GCT GCT GGT CAC GTG ACC CAA GCT CGC ATG GTC	247
Ser Leu Glu Asp Glu Ala Ala Gly His Val Thr Gln Ala Arg Met Val	
55 60 65 70	
AGT AAA AGC AAA GAC GGG ACT GGA AGC GAT GAC AAA AAA GCC AAG GGG	295
Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Asp Lys Lys Ala Lys Gly	
75 80 85	
GCT GAT GGT AAA ACG AAG ATC GCC ACA CCG CGG GGA GCA GCC CCT CCA	343
Ala Asp Gly Lys Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro	
90 95 100	
GGC CAG AAG GGC CAG GCC AAC GCC ACC AGG ATT CCA GCA AAA ACC CCG	391
Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro	
105 110 115	
CCC GCT CCA AAG ACA CCA CCC AGC TCT GGT GAA CCT CCA AAA TCA GGG	439
Pro Ala Pro Lys Thr Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly	
120 125 130	
GAT CGC AGC GGC TAC AGC AGC CCC GGC TCC CCA GGC ACT CCC GGC AGC	487
Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser	
135 140 145 150	
CGC TCC CGC ACC CCG TCC CTT CCA ACC CCA CCC ACC CGG GAG CCC AAG	535
Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Thr Arg Glu Pro Lys	
155 160 165	
AAG GTG GCA GTG GTC CGT ACT CCA CCC AAG TCG CCG TCT TCC GCC AAG	583
Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser Ala Lys	
170 175 180	
AGC CGC CTG CAG ACA GCC CCC GTG CCC ATG CCA GAC CTG AAG AAT GTC	631
Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val	
185 190 195	
AAG TCC AAG ATC GGC TCC ACT GAG AAC CTG AAG CAC CAG CCG GGA GGC	679
Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly	
200 205 210	
GGG AAG GTG CAA ATA GTC TAC AAA CCA GTT GAC CTG AGC AAG GTG ACC	727
Gly Lys Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr	
215 220 225 230	
TCC AAG TGT GGC TCA TTA GGC AAC ATC CAT CAT AAA CCA GGA GGT GGC	775
Ser Lys Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly	
235 240 245	
CAG GTG GAA GTA AAA TCT GAG AAG CTT GAC TTC AAG GAC AGA GTC CAG	823
Gln Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln	
250 255 260	
TCG AAG ATT GGG TCC CTG GAC AAT ATC ACC CAC GTC CCT GGC GGA GGA	871
Ser Lys Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly	
265 270 275	
AAT AAA AAG ATT GAA ACC CAC AAG CTG ACC TTC CGC GAG AAC GCC AAA	919
Asn Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys	
280 285 290	

39

GCC AAG ACA GAC CAC GGG GCG GAG ATC GTG TAC AAG TCG CCA GTG GTG	967
Ala Lys Thr Asp His Gly Ala Glu Ile Val Tyr Lys Ser Pro Val Val	
295 300 305 310	
TCT GGG GAC ACG TCT CCA CGG CAT CTC AGC AAT GTC TCC TCC ACC GGC	1015
Ser Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly	
315 320 325	
AGC ATC GAC ATG GTA GAC TCG CCC CAG CTC GCC ACG CTA GCT GAC GAG	1063
Ser Ile Asp Met Val Asp Ser Pro Gln Leu Ala Thr Leu Ala Asp Glu	
330 335 340	
GTG TCT GCC TCC CTG GCC AAG CAG GGT TG TGATCAGGCC CCTGG	1107
Val Ser Ala Ser Leu Ala Lys Gln Gly	
345 350	

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 351 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ala	Glu	Pro	Arg	Gln	Glu	Phe	Glu	Val	Met	Glu	Asp	His	Ala	Gly	15
1				5					10							
Thr	Tyr	Gly	Leu	Gly	Asp	Arg	Lys	Asp	Gln	Gly	Gly	Tyr	Thr	Met	His	30
			20					25								
Gln	Asp	Gln	Glu	Gly	Asp	Thr	Asp	Ala	Gly	Leu	Lys	Ala	Glu	Glu	Ala	45
			35				40									
Gly	Ile	Gly	Asp	Thr	Pro	Ser	Leu	Glu	Asp	Glu	Ala	Ala	Gly	His	Val	60
			50				55				60					
Thr	Gln	Ala	Arg	Met	Val	Ser	Lys	Ser	Lys	Asp	Gly	Thr	Gly	Ser	Asp	80
			65			70				75						
Asp	Lys	Lys	Ala	Lys	Gly	Ala	Asp	Gly	Lys	Thr	Lys	Ile	Ala	Thr	Pro	95
				85					90							
Arg	Gly	Ala	Ala	Pro	Pro	Gly	Gln	Lys	Gly	Gln	Ala	Asn	Ala	Thr	Arg	110
			100				105									
Ile	Pro	Ala	Lys	Thr	Pro	Pro	Ala	Pro	Lys	Thr	Pro	Pro	Ser	Ser	Gly	125
			115				120									
Glu	Pro	Pro	Lys	Ser	Gly	Asp	Arg	Ser	Gly	Tyr	Ser	Ser	Pro	Gly	Ser	140
			130			135					140					
Pro	Gly	Thr	Pro	Gly	Ser	Arg	Ser	Arg	Thr	Pro	Ser	Leu	Pro	Thr	Pro	160
			145			150				155						
Pro	Thr	Arg	Glu	Pro	Lys	Lys	Val	Ala	Val	Val	Arg	Thr	Pro	Pro	Lys	175
				165					170							
Ser	Pro	Ser	Ser	Ala	Lys	Ser	Arg	Leu	Gln	Thr	Ala	Pro	Val	Pro	Met	190
			180					185								
Pro	Asp	Leu	Lys	Asn	Val	Lys	Ser	Lys	Ile	Gly	Ser	Thr	Glu	Asn	Leu	205
			195					200								

-40-

Lys His Gln Pro Gly Gly Gly Lys Val Gln Ile Val Tyr Lys Pro Val
210 215 220

Asp Leu Ser Lys Val Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His
225 230 235 240

His Lys Pro Gly Gly Gly Gln Val Glu Val Lys Ser Glu Lys Leu Asp
245 250 255

Phe Lys Asp Arg Val Gln Ser Lys Ile Gly Ser Leu Asp Asn Ile Thr
260 265 270

His Val Pro Gly Gly Gly Asn Lys Lys Ile Glu Thr His Lys Leu Thr
275 280 285

Phe Arg Glu Asn Ala Lys Ala Lys Thr Asp His Gly Ala Glu Ile Val
290 295 300

Tyr Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu Ser
305 310 315 320

Asn Val Ser Ser Thr Gly Ser Ile Asp Met Val Asp Ser Pro Gln Leu
325 330 335

Ala Thr Leu Ala Asp Glu Val Ser Ala Ser Leu Ala Lys Gln Gly
340 345 350

41

B

- 41 -

WHAT IS CLAIMED IS:

- sub c1
1. A method for diagnosing, subtyping or monitoring Alzheimer's disease in a patient, comprising assaying a sample of body fluid obtained from the patient for the presence of a tau-peptide using either an anti-tau-peptide antibody or an anti-tau-peptide Fab fragment, wherein further the tau-peptide is derived from proteolytic action on neurofibrillary tangles resulting in fragments from the amino terminal 200 amino acids or carboxy terminal 50 amino acids.
 2. A method for diagnosing, subtyping or monitoring Alzheimer's disease in a patient, comprising assaying a sample of body fluid obtained from the patient for the presence of an anti-tau-peptide autoantibody.
 3. The method for diagnosing, subtyping or monitoring Alzheimer's disease according to claims 1 or 2, wherein the assay is a radioimmunoassay, an enzyme-linked immunosorbant assay, a sandwich assay, a precipitin reaction, a gel immunodiffusion assay, an agglutination assay, a fluorescent immunoassay, a protein A immunoassay or an immunoelectrophoresis assay.
 4. A kit for detecting the presence of a tau-peptide in a sample of body fluid comprising an anti-tau-peptide antibody or an anti-tau-peptide Fab fragment and means for detecting the binding of the tau-peptide with the antibody or fragment.
 5. A kit for detecting the presence of an anti-tau-peptide autoantibody in a sample of body fluid
- sub c2

42

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~~38~~

comprising a tau-peptide and means for detecting the binding of the autoantibody with the tau-peptide.

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Add C3

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SUPPLEMENTAL DECLARATION
AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE the specification of which:

☐ is attached hereto ☒ was filed on August 1, 1991 as Application Serial No. 07/738,778

with amendment(s) filed (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119/§172 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119/172
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint Frank F. Scheck (Reg. No. 17761), S. Leslie Misrock (Reg. No. 18872), Harry C. Jones, III (Reg. No. 20280), Berj A. Terzian (Reg. No. 20060), Gerald J. Flintoft (Reg. No. 20823), David Weild, III (Reg. No. 21094), Jonathan A. Marshall (Reg. No. 24614), Joseph V. Colaianni (Reg. No. 20019), Charles E. McKenney (Reg. No. 22795), Philip T. Shannon (Reg. No. 24278), Barry D. Rein (Reg. No. 22411), Stanton T. Lawrence, III (Reg. No. 25736), Francis E. Morris (Reg. No. 24615), Charles E. Miller (Reg. No. 24576), Gidon D. Stern (Reg. No. 27469), John J. Lauter, Jr. (Reg. No. 27814), Brian M. Poissant (Reg. No. 28462), Brian D. Coggio (Reg. No. 27624), Rory J. Radding (Reg. No. 28749), Stephen J. Harbulak (Reg. No. 29166), Donald J. Goodcell (Reg. No. 19766), James N. Palik (Reg. No. 25510), Thomas E. Friebel (Reg. No. 29258), Laura A. Coruzzi (Reg. No. 30742), Jennifer Gordon (Reg. No. 30753) and Jon R. Stark (Reg. No. 30111), whose address is Pennie & Edmonds, 1155 Avenue of the Americas, New York, New York 10036, and each of them, my attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO: PENNIE & EDMONDS 1155 AVENUE OF THE AMERICAS NEW YORK, N.Y. 10036-2711				DIRE TELEPHONE CALLS TO: PENNIE & EDMONDS (212) 790-9090	
201	FULL NAME OF INVENTOR	LAST NAME Voorheis	FIRST NAME H.	MIDDLE NAME Paul	
	RESIDENCE & CITIZENSHIP	CITY Dublin	STATE OR FOREIGN COUNTRY Ireland	COUNTRY OF CITIZENSHIP USA	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 37 Upper Mount Pleasant Ave.	CITY Rathmines, Dublin 6	STATE OR COUNTRY Ireland	ZIP CODE DEX
202	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
203	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
204	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
205	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
206	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
DATE H. PAUL VOORHEIS 20 September 1991	DATE	DATE
SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
DATE	DATE	DATE

Figure 1

-37 CCGCCTCTGTGACTATCAGGTGAACCTTTGAACAGG

1 Met Ala Glu Pro Arg Gln Glu Phe Glu Val Met Glu Asp His Ala Gly Thr Tyr Gly Leu Gly Asp Arg Lys Asp Gln Gly Gly Tyr Thr
1 ATG GCT GAG CCC GCG CAG GAG TTC GAA GTG ATG GAA GAT CAC GCT GGG ACC TAC GGG TTG GGG GAC AGG AAA GAT CAG GCG GGC TAC ACC
31 Met His Gln Asp Gln Glu Gly Asp Thr Asp Ala Gly Leu Lys Ala Glu Glu Ala Gly Ile Gly Asp Thr Pro Ser Leu Glu Asp Glu Ala
91 ATG CAC CAA GAC CAA GAG GGT GAC ACC GAC GCT GGC CTG AAA GCT GAA GAA GCA GGC ATT GGA GAC ACC CCC AGC CTG GAA GAC GAA GCT
61 Ala Gly His Val Thr Gln Ala Arg Met Val Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Asp Lys Lys Ala Lys Gly Ala Asp Gly Lys
181 GCT GGT CAC GTG ACC CAA GCT CGC ATC GTC AGT AAA AGC AAA GAC GGG ACT GGA AGC GAT GAC AAA AAA GCC AAG GCG GCT GAT GGT AAA
91 Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro Pro Ala
271 ACG AAG ATC GCC ACA CCG CCG GGA GCA GCC CCT CCA GGC CAG AAG GGC CAG GCC AAC GCC ACC AGG ATT CCA GCA AAA ACC CCG CCC GCT
121 Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser
361 CCA AAG ACA CCA CCC AGC TCT GGT GAA CCT CCA AAA TCA GGG GAT CCG AGC GGC TAC AGC AGC CCC GGC TCC CCA GGC ACT CCC GGC AGC
151 Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser
451 CGC TCC CGC ACC CCG TCC CTT CCA ACC CCA CCC ACC CCG GAG CCC AAG AAG GTG GCA GTG GTC CGT ACT CCA CCC AAG TCG CCG TCT TCC
181 Ala Lys Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His
541 GCC AAG AGC CGC CTG CAG ACA GCC CCC GTG CCC ATG CCA GAC CTG AAG AAT GTC AAG TCC AAG ATC GGC TCC ACT GAG AAC CTG AAG CAC
211 Gln Pro Gly Gly Gly Lys Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His
631 CAG CCG GGA GGC GCG AAG GTG CAA ATA GTC TAC AAA CCA GTT GAC CTG AGC AAG GTG ACC TCC AAG TGT GGC TCA TTA GGC AAC ATC CAT
241 His Lys Pro Gly Gly Gly Gln Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser Lys Ile Gly Ser Leu Asp Asn
721 CAT AAA CCA GGA GGT GGC CAG GTG GAA GTA AAA TCT GAG AAG CTT GAC TTC AAG GAC AGA GTC CAG TCG AAG ATT GCG TCC CTG GAC AAT
271 Ile Thr His Val Pro Gly Gly Gly Asn Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala Lys Thr Asp His Gly
811 ATC ACC CAC GTC CCT GGC GGA GGA AAT AAA AAG ATT GAA ACC CAC AAG CTG ACC TTC CGC GAG AAC GCC AAA GCC AAG ACA GAC CAC GGC
301 Ala Glu Ile Val Tyr Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser Ile Asp Met
901 GCG GAG ATC GTG TAC AAG TCG CCA GTG GTG TCT GGG GAC ACG TCT CCA CCG CAT CTC AGC AAT GTC TCC TCC ACC GCG AGC ATC GAC ATG
331 Val Asp Ser Pro Gln Leu Ala Thr Leu Ala Asp Glu Val Ser Ala Ser Leu Ala Lys Gln Gly Leu ***
991 GTA GAC TCG CCC CAG CTC GCC ACG CTA GCT GAC GAG GTG TCT GCC TCC CTG GCC AAG CAG GGT TTG TGA TCAGGCCCTGG



**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
08/159,969	11/30/93	PAUL H. VOORHEIS	4697-059

PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036-2711

EXAMINER	
ART UNIT	PAPER NUMBER
	2

DATE MAILED: 12/21/93

**IF NO RESPONSE TO THIS NOTICE IS RECEIVED WITHIN FORTY-FIVE DAYS, A
FORMAL REQUIREMENT WILL BE ISSUED**

The subject matter of this application appears to:

☒ be "useful in the production or utilization of special nuclear material or atomic energy" as recited in 42 U.S.C. 2182 (Department of Energy (DOE)).

☐ "have significant utility in the conduct of aeronautical and space activities" as recited in 42 U.S.C. 2457 (National Aeronautics and Space Administration (NASA)).

Accordingly, no patent can issue on this application unless applicant(s) file a statement (under oath or in the form of a declaration as provided by 37 CFR 1.68) setting forth (1) the full facts concerning the circumstances under which the invention was made and conceived and (2) the relationship (if any) of the invention to the performance of any work under any contract or other arrangement with the Agency(ies) noted above. On the reverse side of this form is an example of an acceptable format for this statement. The language appearing in paragraphs III and/or IV of the example *must* appear if applicant is attempting to establish that no relationship (under item 2 above) exists.

If the invention disclosed in this application was developed under a contract, grant or cooperative agreement between the Agency indicated above and a person, small business or non-profit organization and rights to the invention have been determined by specific reference to 35 U.S.C. 202 in the contract, grant or cooperative agreement, then applicant need not submit the statement described above. Instead, applicant may file a verified statement (under oath or in the form of a declaration, 37 CFR 1.68) setting forth the information required by 35 U.S.C. 202(c)(6).

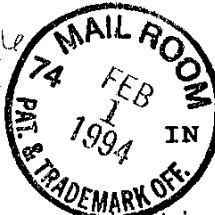
IF NO STATEMENT HAS BEEN RECEIVED WITHIN FORTY-FIVE DAYS OF THE MAIL DATE INDICATED ABOVE, a formal requirement for statement will then be issued. No provision is made for extension of the statutory thirty-day period for response to the formal requirement and the penalty for failure to file an acceptable and timely statement is abandonment of the application. Therefore, applicants are strongly encouraged to submit a statement at this time in order to avoid the issuance of a formal requirement.

IT IS IMPORTANT TO NOTE that the statement must accurately represent the property rights situation of the claimed invention if and when the application is found allowable. Thus, if during prosecution before the examiner, the claimed invention is so altered or the property rights situation so changed as to impact the accuracy of a statement submitted earlier, a supplemental statement must be filed. Failure to submit such additional information where appropriate may be considered a false representation of material facts and render the patent owner vulnerable to loss of patent rights and other sanctions as set forth in the statutes. The PTO will not review allowed applications for this possibility. The responsibility for complying with the statutes rests with the applicants.

Any questions regarding this requirement should be directed to Licensing and Review at (703) 308-3312.

**PLEASE DIRECT ALL COMMUNICATIONS RELATING TO THIS MATTER TO THE
ATTENTION OF LICENSING AND REVIEW**

6570
0740 26



#3
Statement (154)
PATENT
2/28/94

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: VOORHEIS, H.P.

Serial No.: 08/159,969
Filed: November 30, 1993
For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Group Art Unit:
Examiner:
Attorney Docket No: 697-059
Attention: Licensing and
Review

RECEIVED
FEB 1 1994
DEPT. OF ENERGY & REVIEW

**RESPONSE TO NOTICE RELATING TO THE PRODUCTION OR
UTILIZATION OF SPECIAL NUCLEAR MATERIAL OR ATOMIC ENERGY
AS RECITED IN 42 U.S.C. 2182 (DEPARTMENT OF ENERGY (DOE))**

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Enclosed herewith is a Declaration filed in response to a
Notice stating that:

the subject matter of this application
appears to be 'useful in the production or
utilization of special nuclear material or
atomic energy' as recited in 42 U.S.C.
2182 (Department of Energy (DOE)).

Said Declaration sets forth 1) the full facts concerning
the circumstances under which the invention described in the
above-captioned patent application was made and conceived and
2) states that the invention described in the above-captioned
patent application is not related to the performance of any

EXPRESS MAIL CERTIFICATION

"Express Mail" label No. TB389 226 123US Date of Deposit February 1, 1994
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under
37 C.F.R. 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

MICHAEL VIGUJE
(Type or print name of person mailing paper or fee)

(Signature of person mailing paper or fee)

PENY-241817.1

work under any contract or other arrangement with the
Department of Energy (DOE).

Date February 1, 1994

Respectfully submitted,

By Samuel B. Adams 30,605
S. Leslie Misrock 18,872
S. Leslie Misrock (Reg. No.)

PENNIE & EDMONDS
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosure



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
VOORHEIS, H. P.

Serial No.: 08/159,969

Filed: November 30, 1993

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

:
:
Group Art Unit:
:
Examiner:
:
Attention: Licensing and Review
:
:
:

DECLARATION

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

SIR:

I, H. PAUL VOORHEIS, declare and state as follows:

1. That I, H. PAUL VOORHEIS, a citizen of the United States, reside at 37 Upper Mount Pleasant Avenue, Rathmines, Dublin 6, Ireland.
2. That I made and conceived the invention described and claimed in patent application Serial No. 08/159,969, filed on November 30, 1993 entitled "DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE."
3. That in the course of research and investigations carried out during my employment at Trinity College, Dublin 2, Ireland and using the facilities, equipment, materials, funds, information and services of Trinity College, I conceived and made the above-identified invention.
4. That the invention was not made or conceived in the course of, or in connection with, or under the terms of any contract, subcontract or arrangement entered into with or for the benefit of the United States Atomic Energy Commission or its successors: Energy Research and Development Administration or the Department of Energy.

I hereby declare further that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States

Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 24 January 1994

H. Paul Voorheis
H. PAUL VOORHEIS

FD-103c
(Rev. 6-79)
SECURITY
FILE

GROUP NO

FILING DATE

SERIAL NO. (NEWER EDITIONS)

APPLICANT

INVENTION

DRAWINGS

TOTAL CL'S

IND CL'S

FILING FILE REC

TRANSACTION

ATTY. GA

ARMY

NAVY

AIR

CE

DOE

NASA

NSA

claims

159969

#4

ACCESS ACKNOWLEDGEMENT
As Required by

Title 35, United States Code (1952) Section 181

I hereby acknowledge that I have inspected the disclosure of the above identified application for patent in the administration of the law cited above, on behalf of the department or agency which I represent, and promise that any information acquired from said application will not be divulged, disclosed or used for any purpose other than in the administration of the cited law.

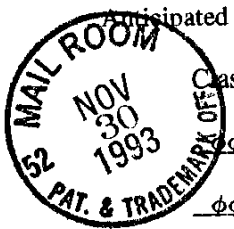
NAME	DATE	AGENCY REPRESENTED
SNR <i>Esamer</i>	3/4/94	DOE



08/159969

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

5/1/93
4/4/94



Assigned Classification of this application:

Class (subclass) 00
00
00

Prior application:

Examiner C. Bidwell

Art Unit 1802

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

This is a request for filing a ☒ continuation ☐ divisional application under 37 CFR § 1.60, of pending prior application serial no. 07/738,778 filed on August 1, 1991

of H. Paul Voorheis
(inventor(s) currently of record in prior application)

for DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE
(title of invention)

1. ☒ Enclosed is a copy of the prior application, including the oath or declaration, as originally filed. I hereby verify that the enclosed is a true copy of the prior application.
2. ☐ Enclosed is a copy of the prior application, including the oath or declaration as originally filed and an affidavit or declaration verifying it as a true copy.
3. ☒ The filing fee is calculated below:

**CLAIMS AS FILED IN THE PRIOR APPLICATION, LESS ANY CLAIMS
CANCELLED BY AMENDMENT BELOW**

EXPRESS MAIL CERTIFICATION

"Express Mail" label No. TB 389 224 908US Date of Deposit November 30, 1993
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Ismael Maldonado
(Signature of person mailing paper or fee)

PENY-227188.1

PENNIE & EDMONDS DOCKET NO. 4697-059

PATENT APPLICATION FEE VALUE

TYPE	NO. FILED	LESS	EXTRA	EXTRA RATE	FEE
Total Claims	5	-20	0	\$22.00 each	0.00
Independent	4	-3	1	\$74.00 each	74.00
Basic Fee					710.00
Multiple Dependency Fee If Applicable (\$230.00)					
Total					784.00
50% Reduction for Independent Inventor, Nonprofit Organization or Small Business Concern					
Total Filing Fee					\$ 784.00

4. ☒ Please charge the required fee to Pennie & Edmonds Deposit Account No. 16-1150. A copy of this sheet is enclosed.
5. ☐ Cancel in this application original claims $\phi\phi$ of the prior application and add new claims $\phi\phi$ by preliminary amendment before calculating the filing fee (At least one original independent claim must be retained for filing purposes).
6. ☒ Amend the specification by inserting before the first line the following sentence: This is a ☒ continuation, of application serial no. 07/738,778, filed August 1, 1991 *now abandoned*
- 7a. ☐ Transfer the drawings from the prior application to this application and abandon the prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file.
- 7b. ☐ New formal drawings are enclosed.
- 7c. ☒ Informal drawings are enclosed.
- 8a. ☐ Priority of application serial no. $\phi\phi$ filed on $\phi\phi$ in $\phi\phi$ is claimed under 35 U.S.C. §119.
- 8b. ☐ The certified copy has been filed in prior application serial no. $\phi\phi$, filed $\phi\phi$.
9. ☐ The prior application is assigned of record to $\phi\phi$.
- 10a. ☒ The Power of Attorney appears in the original papers in the prior application serial no. 07/738,778, filed August 1, 1991.
- 10b. ☐ Since the Power of Attorney does not appear in the original papers, a copy of the Power in prior application serial no. $\phi\phi$, filed $\phi\phi$ is enclosed.

PENY-227188.1

43

PENNIE & EDMONDS DOCKET NO. 4697-059

11. ☒ A preliminary amendment is enclosed.
12. ☐ A preliminary amendment will be filed after issuance of the filing receipt.
- 13a. ☐ Small entity status was established in prior application serial no. $\phi\phi$ filed $\phi\phi$, and is still proper and desired.
- 13b. ☐ A verified statement claiming small entity status is enclosed.
14. ☒ This application contains nucleic acid and/or amino acid sequences required to be disclosed in a Sequence Listing under 37 CFR §§1.821-1.825. It is requested that the Sequence Listing in computer readable form from prior application serial no. 07/738,778, filed on August 1, 1991 be made a part of the present application as provided for by 37 C.F.R. §1.821(e). The sequences disclosed therein are the same as the sequences disclosed in this application. A copy of the paper Sequence Listing from serial no. 07/738,778 is enclosed. Pursuant to 37 C.F.R. §1.821(f), such paper Sequence Listing and such Sequence Listing in computer readable form are the same.
15. ☒ The undersigned states, under 37 C.F.R. §1.821(f), that the content of the enclosed paper Sequence Listing from serial no. 07/738,778 is the same as the content of the computer readable form submitted in application serial no. 07/738,778.
16. ☒ Additional enclosures or instructions. A copy of the Petition For Extension of Time Under 37 C.F.R. § 1.136(a) that was filed in parent application Serial No. 07/738,778 is enclosed herewith. Such petition was filed to ensure copendency of the two applications.
17. ☒ Address all future communications to PENNIE & EDMONDS, 1155 Avenue of the Americas, New York, New York 10036-2711.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Nov. 30, 1993
(date)

By Samuel B. Adams 30,605
S. Leslie Misrock 18,872
S. Leslie Misrock (signature) (Reg No.)

Name and address of signator:

Pennie & Edmonds

1155 Avenue of the Americas

New York, New York 10036-2711

Tel. No. (212) 790-9090

Inventor(s)

Assignee of complete interest

X_____
Attorney or agent of record

Filed under rule 34(a)

PENNIE & EDMONDS

PENY-227188.1



08/159969

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis

Serial No.: Unassigned

Group Art Unit:

Filed:

Examiner:

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

6/18
4/4/94

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicant requests consideration of the following
amendments and remarks.

IN THE SPECIFICATION

Please amend the specification as follows:

At page 12, line 2 after "complete nucleotide"
insert -- (SEQ ID NO:1) --.

At page 12, line 3 after "amino acid sequences"
insert -- (SEQ ID NO:2) --.

After page 36 insert pages 37 to 40 of the Sequence
Listing submitted herewith.

IN THE CLAIMS

Please amend the pagination of the claims as
follows:

EXPRESS MAIL CERTIFICATION

"Express Mail" label No. TB 389 224 968US Date of Deposit November 30, 1993
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under
37 C.F.R. 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

ISMAEL MALDONADO
(Type or print name of person mailing paper or fee)
Ismael Maldonado
(Signature of person mailing paper or fee)

PENY-125138.1

Page 37 to be renumbered as page 41.

Page 38 to be renumbered as page 42.

REMARKS

The specification and claims have been amended to incorporate the sequence identifiers pursuant to 37 C.F.R. §1.821(d) and the Sequence Listing pursuant to §1.821(c), and to renumber the pages of the claims accordingly. It should be noted that the above-made amendments do not constitute new matter under 35 U.S.C. §132.

Applicants respectfully request that the above-made amendments be made of record in the file history of the instant application.

Respectfully submitted,

By *Lamm/B. Adams* 30,605

Date Nov. 30, 1993

S. Leslie Misrock 18,872
S. Leslie Misrock (Reg. No.)

PENNIE & EDMONDS
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

081159969


**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

 Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/159,969	11/30/93	VOORHEIS	H 4697059

18M1/0405

 BIDWELL, E.
EXAMINER

 PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036-2711

ART UNIT	PAPER NUMBER
1802	7

DATE MAILED: 04/05/94

 This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

- ☒ This application has been examined
 ☒ Responsive to communication filed on 11/30/93
☐ This action is made final.

A shortened statutory period for response to this action is set to expire three month(s), _____ days from the date of this letter.
 Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- | | |
|---|--|
| 1. ☒ Notice of References Cited by Examiner, PTO-892. | 2. ☒ Notice re Patent Drawing, PTO-948. |
| 3. ☐ Notice of Art Cited by Applicant, PTO-1449. | 4. ☐ Notice of Informal Patent Application, Form PTO-152. |
| 5. ☐ Information on How to Effect Drawing Changes, PTO-1474. | 6. ☐ _____ |

Part II SUMMARY OF ACTION

1. ☒ Claims 1-5 are pending in the application.

Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☒ Claims 1-5 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ disapproved (see explanation).

12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received.
☐ been filed in parent application, serial no. _____; filed on _____

13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

EXAMINER'S ACTION

Serial Number: 08/159,969
Art Unit: 1802

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The preliminary amendments filed November 30, 1993 have been entered into the record which inserted SEQ ID NOS into the specification, inserted the paper copy of SEQ ID NO:1 and SEQ ID NO:2 into the specification and renumbered the pages accordingly. Claims 1-5 are pending.

The disclosure is objected to because of the following informalities: the status of the parent application inserted by preliminary amendment into the specification before line 1 should be updated. Appropriate correction is required.

35 U.S.C. § 101 reads as follows:

"Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title".

Claims 1-5 are rejected under 35 U.S.C. § 101 because the claimed invention is inoperative and thus lacks patentable utility.

Claims 1-3 are directed to methods for diagnosing, subtyping or monitoring Alzheimer's disease in a patient and claims 4 and 5 are directed to a kit for detecting the presence of a tau-peptide in a sample and a kit for detecting the presence of an anti-tau peptide autoantibody in a sample. To date the only definitive diagnosis of Alzheimer's disease can be made through postmortem examination of the brain where gross pathological changes are found in the brain as set forth in the specification on page 3-4 (also see Foley et al, J. Neurol., 1988). There is no evidence of record that shows a positive correlation between detection of tau-peptide in a sample of body fluid or detection of anti-tau-peptide autoantibody in a sample of body fluid with a confirmed, definitive diagnosis of Alzheimer's disease in that patient. Because the only utility for the test kits is for use in diagnosing Alzheimer's disease, the kits like utility for the same reasons that the methods do. Moreover, if a patient cannot be definitively diagnosed until after death, it follows that the disease cannot be "monitored" at that point. There is no evidence of record that a change in the amount of detected tau-peptide or anti-tau-peptide autoantibody in a sample of body fluid correlates

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with a change in cognitive abilities of the patient so as to allow for the disease to be monitored nor is there any evidence that by detecting either the peptide or the autoantibody it is possible to "subtype" the particular form of Alzheimer's disease in that patient. Absent evidence which establishes these particular aspects of the claimed invention, the methods and kits are deemed to lack utility.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to adequately describe and/or provide an enabling disclosure for the invention being claimed.

The specification fails to teach or describe how to diagnose, subtype or monitor Alzheimer's disease by detecting the presence of a tau-peptide or the presence of an anti-tau-peptide autoantibody in a sample of body fluid obtained from a patient. As discussed above, Alzheimer's disease can only be definitively diagnosed by examining the brain. Patients suffering from dementia can be evaluated using clinical and neuropsychological measurements and assessed as having "probable" or "possible" Alzheimer's disease under guidelines established by the CERAD although again the validity of the diagnosis must await confirmation by longitudinal observations and ultimately by histopathologic examination (see Morris et al, Neurology, 1989). The specification does not teach that clinically diagnosed as "probable" or "possible" Alzheimer's disease patients who have tau-peptide or anti-tau-peptide autoantibody present in a sample of body fluid subsequently have been definitively diagnosed as having Alzheimer's disease using the recognized criteria. Thus the methods disclosed appear to be directed to screening methods which aid in diagnosing of Alzheimer's disease. Further the specification fails to teach how to monitor Alzheimer's disease using the

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claimed methods because if the diagnosis of Alzheimer's disease cannot be made until after death, the disease at that point cannot be monitored. If the method was limited to a screening method, the specification does not teach or describe that a change in the amount of either marker has any effect on the progression of the disease, e.g., that a decrease in either marker correlates with an improvement in the cognitive skills for the patient being monitored or that an increase correlates with a deterioration of the mental state of the patient. The specification does not teach or describe how to subtype Alzheimer's disease using the claimed methods because it does not set forth any guidelines for correlating detection of *tau*-peptide or anti-*tau*-peptide autoantibody with recognized subtypes of Alzheimer's disease, e.g., familial Alzheimer's disease. The specification does not teach how to assay a sample of body fluid for the presence of a *tau*-peptide where the *tau*-peptide is derived from proteolytic action on neurofibrillary tangles resulting in fragments from the amino terminal 200 amino acids or carboxyl terminal 50 amino acids using either an anti-*tau*-peptide antibody or Fab fragment thereof. By using an antibody or Fab fragment thereof, both intact *tau* as well as proteolytic fragments of *tau* peptide will bind to those antibodies recognizing the terminal regions of the intact *tau* and thus one cannot distinguish the intact *tau* protein from proteolytic fragments using only one antibody. The specification does not teach how to perform the immunoassay methods recited in claim 3 for detecting the *tau*-peptide because it does not teach or disclose which other reagents can be used in those assay to distinguish the *tau*-peptide from the intact *tau* protein. The specification does not teach how to assay a sample of body fluid for the presence of an anti-*tau*-peptide autoantibody without the use of any specific binding reagents in the method being claimed. Thus without further guidance to these aspects, it would take an undue amount of experimentation to practice the invention being claimed.

Claims 1-5 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

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Claims 1-3 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 2 and claim 3 dependent on each are vague and indefinite because the preamble of claims 1 and 2 are directed to a method of diagnosing, subtyping or monitoring Alzheimer's disease and yet neither claim includes a correlation step to accomplish these goals.

Claim 3 is vague because "the assay" lacks antecedent basis in claim 1 or claim 2 from which it depends.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --
(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 3 and 4 are rejected under 35 U.S.C. § 102(b) as being anticipated by Wolozin et al (Ann. Neurol., 22:521-526, 1987) in light of Ksiezak-Reding et al (J. Neurosci. Res., 25:412-419). Wolozin et al disclose monoclonal antibody Alz-50 recognizes a protein which they call A68 in the brains of patients who died with Alzheimer's disease but not in the brain tissue of individuals free of neurological disease and that A68 can readily be detected in CSF using Western blot analysis with labelled goat anti-mouse immunoglobulin as the detection reagent (page 522, col. 2, first full paragraph). Wolozin et al disclose the usefulness of detection of A68 in CSF for aiding in diagnosing Alzheimer's disease including a sandwich immunoassay (Abstract on page 521 and page 526, first paragraph). Ksiezak-Reding et al disclose that the Alz 50 epitope is located at the N-terminus of tau between Tau 60 and Tau 14 epitopes (Abstract on page 412). Because Ksiezak-Reding show that the Alz-50 antibody binds to the N-terminal region of tau, Wolozin et al disclose the method and kit being instantly claimed.

Claim 4 is rejected under 35 U.S.C. § 102(b) as being anticipated by Kosik et al (Neuron, 1:817-825, Nov. 1988). Kosik et al disclose a collection

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of components including 10 different monoclonal and four different polyclonal antibodies that react with *tau* on immunoblots using anti-IgG conjugated with alkaline phosphatase as the detection reagent (page 823, Tau Protein and Antibodies).

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 1, 3 and 4 are rejected under 35 U.S.C. § 103 as being unpatentable over Wolozin et al (Ann. Neurol., 22:521-526, 1987) in combination with Ksiezak-Reding et al (J. Neurosci. Res., 25:412-419).

Wolozin et al is set forth above but differs by using the Alz 50 antibody which Ksiezak-Reding et al disclose as recognizing the N-terminal of *tau* and by using intact Alz 50.

Ksiezak-Reding et al is set forth above and further disclose that Alz 50 and seven other monoclonal antibodies have been shown to react with both *tau* and Alzheimer brain proteins of molecular mass 60-70 kDa. Ksiezak-Reding et al disclose other monoclonal antibodies which bind to specific epitopes on the *tau* protein at residues 60-72, 83-120, 131-140 and 315-353 (Abstract on page 412) but differ by not using other anti-*tau* monoclonal antibodies to detect the presence of *tau* in samples of body fluid.

It would have been obvious to one of ordinary skill in the art to test a sample of body fluid for the presence of *tau* as an aid for Alzheimer's disease as taught by Wolozin et al by using other antibodies which bind to recognize N-terminal 200 amino acids because Wolozin teaches that Alz-50 monoclonal antibody can be used to detect the presence of A68 antigen in CSF as a means

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for diagnosing Alzheimer's disease and Ksiezak-Reding teaches that the Alz-50 antibody binds to an epitope on tau located at the N-terminal and they teach that other anti-tau antibodies bind to epitopes near the Alz 50 epitope. Thus one would have reasonably expected to successfully detect tau in body fluids using Ksiezak-Reding's monoclonal antibodies as alternate antibodies requiring only routine screening to determine other useful antibodies which bind to tau because Ksiezak-Reding teaches that Wolozin's Alz 50 binds an epitope near the N-terminal and Ksiezak-Reding teaches that other antibodies bind to epitopes near the epitope of Wolozin's Alz 50 antibody. One of ordinary skill in the art would have been motivated to use Fab fragments of the Alz 50 antibody or Ksiezak-Reding's anti-tau antibodies in place of intact antibodies as a means of improving specificity because Fab fragments have the same antigen-binding specificity as intact antibodies but do not have the Fc region which can cause non-specific binding in an immunoassay. It would have been obvious to package the reagents including the anti-tau Fab fragment or Ksiezak-Reding's anti-tau antibodies along with detection reagents in a kit because kits are convenient and economical means of assembling test components for the user.

Claims 2, 3 and 5 are rejected under 35 U.S.C. § 103 as being unpatentable over Singh et al (Immunol. Lett., 1986) in combination with Foley et al (J. Neurol., 1988), Gaskin et al (Clin. Res., 1986) and Grundke-Iqbal et al (J. Biol.Chem., 261(13):6084-6089, 1986).

Singh et al disclose detection of brain autoantibodies in serum from patients with Alzheimer's disease (AD). Singh et al disclose that AD may be a syndrome with different sub-types of disease, one of which may involve defective immune regulation and/or autoimmunity (page 277, Introduction). Singh et al disclose testing serum for the presence of anti-brain auto-antibodies using brain tissue in an indirect immunofluorescent detection method with goat anti-human FITC conjugate (page 277, second column, first full paragraph). Singh et al disclose that specific staining patterns were found in about 57% of the AD sera whereas none of the 28 normal human sera

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were positive for specific staining. Singh et al do not characterize antigen-binding specificity of the anti-brain autoantibodies detected.

Foley et al disclose data which supports the hypothesis that autoimmune mechanisms may operate in the pathogenesis of Alzheimer's disease. Foley et al disclose that the presence of amyloid deposits and cortical plaques in post-mortem brain tissue has suggested a link between autoimmune responses and the pathogenesis of disease because a blood-brain barrier breakdown has been reported in many cases and because the amyloid deposits and cortical plaques are also found associated with blood vessels. Foley et al further disclose that antibodies which inhibit choline acetyltransferase activity and antibodies which specifically bind to cholinergic neurons have been detected in sera of patients with Alzheimer's disease and that they detect the presence of antibodies which are able to lyse mammalian synaptosomes in sera from groups of patients with Alzheimer's disease (see Abstract and first full paragraph on page 466). Foley et al disclose that researchers have found IgG antibodies in sera of American patients with Alzheimer's disease which are reactive with magnocellular neurons in rat and human basal forebrains detectable using immunocytochemistry and that others have reported the presence of antibodies which specifically recognize cholinergic neurons in CSF of patients with Alzheimer's disease. Still others have detected anti-cholinergic antibodies in sera of patients with Alzheimer's disease using ELISA tests and immunoblotting employing specific cholinergic antigens (page 470, first column, second full paragraph). Foley et al differ by detecting autoantibodies to other antigens.

Gaskin et al disclose that patients clinically diagnosed as having senile dementia of the Alzheimer type (SDAT) make autoantibodies which react with neurofibrillary tangles (NFT) and that other researchers have demonstrated the presence of serum anti-brain antibodies. Gaskin et al disclose establishing B cell lines by Epstein-Barr viral transformation of a limited number of B cells isolated from patients with SDAT where the B cell lines secrete antibodies. The antibodies are screened for isolation of those

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which bind to NFT using indirect immunofluorescence. The preliminary evidence indicates that these antibodies are limited to SDAT patients thus supporting the hypothesis that autoimmunity is a pathogenic factor in SDAT (see entire abstract).

Grundke-Iqbal et al disclose purification of microtubule-associated protein tau and they disclose that tau fractions react specifically with antibodies raised against paired helical filaments from Alzheimer's disease patients (see Abstract on page 6084). Grundke-Iqbal et al disclose that paired helical filaments (PHF) are the most characteristic cytoskeletal alteration affecting neurons in Alzheimer's disease where PHF form neurofibrillary tangles in neuronal perikaryon in dystrophic neurites of the neuritic (senile) plaques in Alzheimer cerebrum. Grundke-Iqbal et al electrophoretically separate tau polypeptides on an SDS-polyacrylamide slab gel and immunolabeled the various bands with antibodies to tau (see Fig. 2 on page 6087). Grundke-Iqbal et al differ by not detecting anti-tau-peptide-autoantibodies.

It would have been obvious to one of ordinary skill in the art to detect autoantibodies to tau protein in body fluid samples by using the various tau fragments of Grundke-Iqbal et al as the means for binding anti-tau-auto-antibodies along with labelled anti-human antibodies as taught by Singh as the detection reagent because Singh et al teach that anti-brain antibodies are detectable in sera of AD patients using anti-human conjugated antibodies, Foley et al teach a link between autoimmune responses and the pathogenesis of Alzheimer's disease via a blood-brain barrier breakdown and detection of antibodies to cholinergic neurons in body fluids from Alzheimer's disease patients using conventional immunoassay methods, Gaskin et al teach the detection of autoantibodies which react with neurofibrillary tangles (NFT) using immunological detection methods and Grundke-Iqbal et al teach that tau protein is a component of paired helical filaments which make up NFT. In the absence of unexpected results one of ordinary skill in the art would have reasonably expected to detect the presence of anti-tau autoantibodies as

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alternates to Singh's anti-brain antibodies or Gaskin's autoantibodies which bind to in sera or CSF samples using the tau fractions of Grundke-Iqbal (equivalent to tau-peptides as instantly claimed) in a variety of conventional immunoassay techniques because Grundke-Iqbal teaches that tau protein is a component of Alzheimer paired helical filaments. It would have been obvious to package the assay reagents including Grundke-Iqbal's tau fractions and means for detecting the autoantibody in a kit because kits are convenient and economical means of assembling test components for the user.

References made of record during prosecution of the parent application have been indicated with an asterisk on the PTO-892.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carol Bidwell whose telephone number is (703) 308-3982.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center numbers are (703) 305-3014 and (703) 308-4227.

cb
April 2, 1994

Carol E Bidwell

CAROL E. BIDWELL
PATENT EXAMINER
GROUP 1800

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP--APART AND DISCARD CARBON

1 of 2

FORM PTO-892 (REV. 3-78)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. 08/159 969	GROUP/ART UNIT 1802	ATTACHMENT TO PAPER NUMBER 7				
NOTICE OF REFERENCES CITED 4/4				APPLICANT(S) Voorheis						
U.S. PATENT DOCUMENTS										
*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE			
	A									
	B									
	C									
	D									
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FOREIGN PATENT DOCUMENTS										
*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG.		PP. SPEC.
	L									
	M									
	N									
	O									
	P									
	Q									
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)										
*	R	Foley et al, "Evidence for the presence of antibodies to cholinergic neurons in the serum of patients with Alzheimer's disease", J. Neurol., 235: 466-471 (1988).								
*	S	Morris et al, "The Consortium to Establish a Registry for Alzheimer's Disease (CERAD)", Neurology 39:1159-1165 (1989).								
	T	Wolozin et al, "Alzheimer-Related Neuronal Protein A68: specificity and Distribution", Ann. Neurol., 22: 521-526 (1987).								
	U	Ksiezak-Reding et al, "Mapping of the A12-50 Epitope in Microtubule-Associated Proteins Tau", J. Neurosci. Res., 25: 412-419 (1990).								
EXAMINER Carol Bedwell			DATE 4/2/94							
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)										

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

2 of 2

FORM PTO-892 (REV. 3-78)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. 08/159969		GROUP/ART UNIT 1802		ATTACHMENT TO PAPER NUMBER 7						
NOTICE OF REFERENCES CITED 4/4				APPLICANT(S) Voorheis										
U.S. PATENT DOCUMENTS														
*		DOCUMENT NO.						DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE		
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	L													
	M													
	N													
	O													
	P													
	Q													
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)														
	R	Kosik et al, "Epitopes That Span the Tau Molecule Are shared with Paired Helical Filaments", <i>Neuron</i> 1: 817-825 (Nov. 1988).												
	S	Singh et al, "Detection of Brain Autoantibodies in the Serum of Patients With Alzheimer's Disease But Not Down's Syndrome", <i>Immunol. Lett.</i> , 12: 277-280 (1986).												
	T	Gasken et al, "Patients with Clinically Diagnosed Senile Dementia of the Alzheimer Type (SDAT) Make Autoantibodies That React with Neurofibrillary Tangles", <i>Clin. Res.</i> , 34(2): 669A (1986).												
	U	Grundke-Iqbal et al, "Microtubule-associated Protein Tau", <i>J. Biol. Chem.</i> , 261(13): 6084-6089 (May 5, 1986).												
EXAMINER Carol Bedwell								DATE 4/2/94						
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)														

81159969

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

PTO Draftpersons review all originally filed drawings regardless of whether they are designated as formal or informal. Additionally, patent Examiners will review the drawings for compliance with the regulations. Direct telephone inquiries concerning this review to the Drawing Review Branch, 703-305-8404.

The drawings filed (insert date) 11/30/93, are
 A. ☒ not objected to by the Draftsperson under 37 CFR 1.84 or 1.152.
 B. ☒ objected to by the Draftsperson under 37 CFR 1.84 or 1.152 as indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawings must be submitted according to the instructions on the back of this Notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings:

Black ink. Color.

☐ Not black solid lines. Fig(s) _____☐ Color drawings are not acceptable until petition is granted.

2. PHOTOGRAPHS. 37 CFR 1.84(b)

☐ Photographs are not acceptable until petition is granted.

3. GRAPHIC FORMS. 37 CFR 1.84 (d)

☐ Chemical or mathematical formula not labeled as separate figure. Fig(s) _____☐ Group of waveforms not presented as a single figure, using common vertical axis with time extending along horizontal axis. Fig(s) _____☐ Individual waveform not identified with a separate letter designation adjacent to the vertical axis. Fig(s) _____

4. TYPE OF PAPER. 37 CFR 1.84(e)

☐ Paper not flexible, strong, white, smooth, nonshiny, and durable. Sheet(s) _____☐ Erasures, alterations, overwritings, interlineations, cracks, creases, and folds not allowed. Sheet(s) _____

5. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable paper sizes:

21.6 cm. by 35.6 cm. (8 1/2 by 14 inches)

21.6 cm. by 33.1 cm. (8 1/2 by 13 inches)

21.6 cm. by 27.9 cm. (8 1/2 by 11 inches)

21.0 cm. by 29.7 cm. (DIN size A4)

☐ All drawing sheets not the same size. Sheet(s) _____☐ Drawing sheet not an acceptable size. Sheet(s) _____

6. MARGINS. 37 CFR 1.84(g): Acceptable margins:

Paper size

21.6 cm. X 35.6 cm. (8 1/2 X 14 inches)	21.6 cm. X 33.1 cm. (8 1/2 X 13 inches)	21.6 cm. X 27.9 cm. (8 1/2 X 11 inches)	21.0 cm. X 29.7 cm. (DIN Size A4)
T 5.1 cm. (2")	2.5 cm. (1")	2.5 cm. (1")	2.5 cm.
L .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")
R .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")
B .64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")	.64 cm. (1/4")

Margins do not conform to chart above.

Sheet(s) _____

☐ Top (T) ☒ Left (L) ☒ Right (R) ☐ Bottom (B)

7. VIEWS. 37 CFR 1.84(h)

REMINDER: Specification may require revision to correspond to drawing changes.

☐ All views not grouped together. Fig(s) _____☐ Views connected by projection lines. Fig(s) _____☐ Views contain center lines. Fig(s) _____

Partial views. 37 CFR 1.84(h)(2)

☐ Separate sheets not linked edge to edge. Fig(s) _____☐ View and enlarged view not labeled separately. Fig(s) _____☐ Long view relationship between different parts not clear and unambiguous. 37 CFR 1.84(h)(2)(ii) Fig(s) _____

Sectional views. 37 CFR 1.84(h)(3)

☐ Hatching not indicated for sectional portions of an object. Fig(s) _____☐ Hatching of regularly spaced oblique parallel lines not spaced sufficiently. Fig(s) _____☐ Hatching not at substantial angle to surrounding axes or principal lines. Fig(s) _____☐ Cross section not drawn same as view with parts in cross section with regularly spaced parallel oblique strokes. Fig(s) _____☐ Hatching of juxtaposed different elements not angled in a different way. Fig(s) _____

Alternate position. 37 CFR 1.84(h)(1)

☐ A separate view required for a moved position. Fig(s) _____

Modified forms. 37 CFR 1.84(h)(5)

☐ Modified forms of construction must be shown in separate views. Fig(s) _____

8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)

☐ View placed upon another view or within outline of another. Fig(s) _____☐ Words do not appear in a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) _____

9. SCALE. 37 CFR 1.84(k)

☐ Scale not large enough to show mechanism without crowding when drawing is reduced in size to two-thirds in reproduction. Fig(s) _____☐ Indication such as "actual size" or "scale 1/2" not permitted. Fig(s) _____☐ Elements of same view not in proportion to each other. Fig(s) _____

10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(l)

☐ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (except for color drawings). Fig(s) _____

11. SHADING. 37 CFR 1.84(m)

☐ Shading used for other than shape of spherical, cylindrical, and conical elements of an object, or for flat parts. Fig(s) _____☐ Solid black shading areas not permitted. Fig(s) _____

12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)

☐ Numbers and reference characters not plain and legible. 37 CFR 1.84(p)(1) Fig(s) _____☐ Numbers and reference characters used in conjunction with brackets, inverted commas, or enclosed within outlines. 37 CFR 1.84(p)(1) Fig(s) _____☐ Numbers and reference characters not oriented in same direction as the view. 37 CFR 1.84(p)(1) Fig(s) _____☐ English alphabet not used. 37 CFR 1.84(p)(2) Fig(s) _____☒ Numbers, letters, and reference characters do not measure at least .32 cm. (1/8 inch) in height. 37 CFR(p)(3) Fig(s) _____

13. LEAD LINES. 37 CFR 1.84(q)

☐ Lead lines cross each other. Fig(s) _____☐ Lead lines missing. Fig(s) _____☐ Lead lines not as short as possible. Fig(s) _____

14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(t)

☐ Number appears in top margin. Fig(s) _____☐ Number not larger than reference characters. Fig(s) _____☐ Sheets not numbered consecutively, and in Arabic numerals, beginning with number 1. Sheet(s) _____

15. NUMBER OF VIEWS. 37 CFR 1.84(u)

☐ Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) _____☐ View numbers not preceded by the abbreviation Fig. Fig(s) _____☐ Single view contains a view number and the abbreviation Fig. Fig(s) _____☐ Numbers not larger than reference characters. Fig(s) _____

16. CORRECTIONS. 37 CFR 1.84(w)

☐ Corrections not durable and permanent. Fig(s) _____

17. DESIGN DRAWING. 37 CFR 1.152

☐ Surface shading shown not appropriate. Fig(s) _____☐ Solid black shading not used for color contrast. Fig(s) _____ATTACHMENT TO PAPER NO. 7REVIEWER WJ

DATE

1/27/94

PTO Copy

Copy 182#8
10-27-94



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, H. Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: Bidwell, C.

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

DECLARATION OF DAVIS COAKLEY, M.D., UNDER 37 C.F.R. § 1.131

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

RECEIVED
OCT 25 1994
GROUP 1800

I, DAVIS COAKLEY DECLARE AS FOLLOWS:

1. I am a citizen of the Republic of Ireland residing at 57 Mount Anville, Dublin 14, Ireland.
2. I received M.B., B.Ch., B.A.O. (the equivalent to an American M.D.) degree in 1971 and an M.D. degree in 1978 from the National University of Ireland. I have been, since 1991, President of the Irish Gerontological Society; since 1984, a Fellow of the Royal College of Physicians in Ireland; and, since 1994, a Fellow of the Royal College of Physicians (London). A copy of my *curriculum vitae* is attached hereto as Exhibit A.
3. My medical practice is conducted at St. James Hospital, Dublin. I have been, since 1979, a Consultant Physician in geriatric medicine, St. James Hospital, Dublin, Ireland and, since 1988, the Director of Mercer's Institute for Research on Ageing at the same institution. I am an Associate Professor of Geriatric Medicine at Trinity College, Dublin, Ireland and Dean of Health Sciences at Trinity College.

CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 17, 1994.

Daniel Haysburg
(Name)

(Signature)

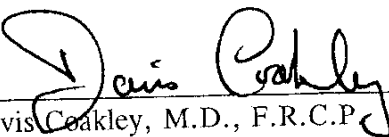
36,156
(Reg. No.)

October 17, 1994
(Date of Signature)

PENY2-305745.1

4. I personally possess no interest in the above-captioned patent application (the Application).
5. The above-identified inventor of the Application (the Inventor) is a member of the faculty of the Department of Biochemistry, Trinity College, Dublin, Ireland. I and several of my medical colleagues, under my supervision, have made available to the Inventor samples of blood from eighteen (18) patients who we had diagnosed as having Alzheimer's Disease. For comparison 18 "control" samples from patients with other diagnoses were provided.
6. The clinical diagnosis of Alzheimer's Disease was made by trained staff working at St. James Hospital. The criteria that are employed are the criteria set in the publication: McKhann, G. et al., 1984, "Clinical Diagnosis of Alzheimer's Disease: Report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Taskforce on Alzheimer's Disease," *Neurology* 34:939-944 (Attached hereto as Exhibit B).
7. These diagnostic criteria, generally referred to as the NINCDS criteria, are generally recognized in the field to be the most acceptable criteria by which to define Alzheimer's Disease.
8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and any patent issuing thereon.

Date: 10.10.94


Davis Coakley, M.D., F.R.C.P.



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, H. Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: C. Bidwell

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

SUPPLEMENTAL REPLY UNDER RULE 111

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Along with the Amendment Under Rule 115, mailed October 5, 1994, Applicant submitted an unexecuted declaration of Dr. Davis Coakley. Thus, Applicant transmits herewith an executed Declaration Submitted Under Rule 132 of Dr. Davis Coakley.

Applicant notes that the executed declaration differs from the unexecuted declaration. The unexecuted declaration may have erroneously created the impression that Dr. Coakley actively participated in planning and executing the research program of the Applicant. Thus, paragraph 5 of the executed declaration has been amended by the deletion of the sentence that states that Dr. Coakley was a collaborator and assistant of the Applicant.

Date 17. October 1994

Respectfully submitted,

S. Leslie Misrock

102 Hery 36,156 18,872
S. Leslie Misrock (Reg. No.)
PENNIE & EDMONDS
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosure

CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 17, 1994.

Daniel Hansburg
(Name)

102 Hery
(Signature)

36,156
(Reg. No.)

October 17, 1994
(Date of Signature)

PENY2-312670.1



GRP/802
PATENT #9
10/13/94

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: C. Bidwell

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

RECEIVED
OCT 2 1994

INFORMATION DISCLOSURE STATEMENT
UNDER 37 C.F.R. §§ 1.56 AND 1.97

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

In accordance with Applicants' duty of disclosure imposed by 37 C.F.R. §§ 1.56 and 1.97(c), Attorneys for Applicants hereby invite the Examiner's attention to the references cited in the attached form. Photocopies of the cited references are submitted herewith.

Identification of the submitted references is not to be construed as an admission of Applicants or Attorneys for Applicants that such references are available as "prior art" against the subject application. Consequently, Applicants respectfully decline to use form PTO-1449, since this form identifies all of the references therein as "Prior Art." As an alternative, Applicants submit herewith a "revised PTO Form 1449" entitled "List of References Cited" instead of "List of Prior Art Cited." Applicant respectfully requests

P 30567 10/24/94 08159969 16-1150 030 126 210.00CH

CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 5, 1994.

Daniel Hansburg
(Name)


(Signature)

36,156

(Reg. No.)

October 5, 1994
(Date of Signature)

PENY2-309631.1

that the listed and enclosed references be considered by the Examiner and made of record in the above-identified application.

Pursuant to 37 C.F.R. §1.97(c), this Information Disclosure Statement is filed after the mailing date of the First Office Action on the merits; therefore a fee of \$210.00 is required pursuant to 37 C.F.R. § 1.17(p). A separate Fee Transmittal Letter authorizing payment of this fee is attached hereto.

Respectfully submitted,

S. Leslie Misrock

Date October 5, 1994

by *WLL JHJ* 36156 18,872
S. Leslie Misrock (Reg. No.)
PENNIE & EDMONDS
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090



LIST OF REFERENCES CITED BY APPLICANT <i>(Use several sheets if necessary)</i>					ATTY. DOCKET NO. 4697-059		SERIAL NO. 08/159,969	
					APPLICANT Voorheis, Paul			
					FILING DATE November 30, 1993		GROUP 1802	
U.S. PATENT DOCUMENTS								
*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE	
FOREIGN PATENT DOCUMENTS								
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION	
							YES NO	
PAD	AA	1,302,250	6-2-92	Canada				
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)								
PAD	AB	Ksiezak-Reding et al., 1988, "Immunochemical and biochemical characterization of τ proteins in normal and Alzheimer's disease brains with Alz 50 and Tau-1", J. Biol. Chem. 263(17):7948-7953						
PAD	AC	Kingsley et al., 1988, "Human antibodies to neurofibrillary tangles and astrocytes in Alzheimer's disease", J. Neuroimmunol. 19:89-99						
PAD	AD	Gaskin et al., 1987, "Autoantibodies to neurofibrillary tangles and brain tissue in Alzheimer's disease: Establishment of Epstein-Barr Virus-transformed antibody-producing cell lines", J. Exp. Med. 165:245-250						
PAD	AE	Iqbal et al., 1989, "Laboratory Diagnostic tests for Alzheimer's disease", Prog. Clin. Biol. Res. 317:679-687						
EXAMINER <i>PATRICIA A. DUFFY</i>					DATE CONSIDERED <i>January 9, 1995</i>			
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.								



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: C. Bidwell

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

FEE TRANSMITTAL SHEET

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

The fee required to be filed with the accompanying Information Disclosure Statement Under C.F.R. §§ 1.56 and 1.97(c) of even date herewith concerning the above-identified patent application is believed to be \$210.00 pursuant to 37 C.F.R. § 1.17(p).

Please charge this fee to Pennie & Edmonds Deposit Account No. 16-1150. However, should any additional fee be required, the Commissioner is authorized to charge any such fee or credit any overpayment to Deposit Account No. 16-1150.

A copy of this sheet is enclosed.

Respectfully submitted,

S. Leslie Mirock

Date October 5, 1994

by 10/7/94 36156 18,872
S. Leslie Mirock (Reg. No.)
PENNIE & EDMONDS
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 5, 1994.

Daniel Hansburg
(Name)

[Signature]
(Signature)

36,156
(Reg. No.)

October 5, 1994
(Date of Signature)

PENY2-309698.1



Corp 1802
PATENT #10
11/3/94

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
WOOD, H. Paul

Serial No.: 08/159,969

Filed: November 30, 1993

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Group Art Unit: 1802

Examiner: C. Bidwell

Attorney Docket No.: 4697-059

193 10/27

PETITION FOR EXTENSION OF TIME UNDER 37 CFR § 1.136(a)

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

It is respectfully requested that the time for response to the Office Action dated April 5, 1994, be extended for a period of 3 month(s) from July 5, 1994 to and including October 5, 1994.

The fee for this extension is estimated to be \$ 870.00. Please charge the required fee to Pennie & Edmonds Deposit Account No. 16-1150. A copy of this sheet is enclosed.

Respectfully submitted,
S. Leslie Misrock

Date October 5, 1994

by *[Signature]* 36,156 18,872
S. Leslie Misrock (Reg. No.)
PENNIE & EDMONDS

1155 Avenue of the Americas
New York, N.Y. 10036-2711
(212) 790-9090

P-30566 10/24/94 08159969 16-1150 030 117 870.00CH
CERTIFICATE UNDER 37 C.F.R. 1.87(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 5, 1994.

Daniel Hansburg *[Signature]* 36,156 October 5, 1994
(Name) (Signature) (Reg. No.) (Date of Signature)

PENY2-309562.1

#11/c
JW 11/3/94



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, H. Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: Bidwell, C.

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

RECEIVED

OCT 26 1994

GROUP 1802

AMENDMENT UNDER 37 C.F.R. § 1.115

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Please consider the following amendments and remarks, which are responsive to the Office Action mailed April 5, 1994. Enclosed herewith are Declarations Submitted Under Rule 132 by the Drs. Davis Coakley (unexecuted) and H. Paul Voorheis, a Petition to Extend Time Under 37 C.F.R. § 1.136(a) and an Information Disclosure Statement submitted according to Rule 1.97(c), along with provision for payment of the applicable fees.

AMENDMENTS

In the Specification:

Please amend the first sentence of the specification as follows:

This is a continuation of application Serial No. 07/738,778, filed August 1, 1991, now abandoned.

CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 5, 1994.

Daniel Hansburg
(Name)

(Signature)

36,156
(Reg. No.)

October 5, 1994
(Date of Signature)

PENY2-304567.1

In the Claims:

✓ Please cancel claim 4, without prejudice. Please amend the claims as follows:

Sub D1
C1
1. (amended) A method for diagnosing[, subtyping or monitoring] Alzheimer's disease in a patient suspected of having Alzheimer's Disease, comprising assaying a sample of [body fluid] blood or cerebrospinal fluid obtained from the patient for [the presence] a concentration of a tau-peptide using either an anti-tau-peptide antibody or an anti-tau-peptide Fab fragment, wherein further the tau-peptide is derived [from proteolytic action on neurofibrillary tangles resulting in fragments] from the amino terminal 200 amino acids or carboxy terminal 50 amino acids of a tau-protein; and employing the assayed concentration to evaluate the status of the patient's Alzheimer's Disease.

2. (amended) A method for diagnosing[, subtyping or monitoring] Alzheimer's disease in a patient suspected of having Alzheimer's Disease, comprising assaying a sample of [body fluid] blood or cerebrospinal fluid obtained from the patient for [the presence] a concentration of an anti-tau-peptide autoantibody; and employing the assayed concentration to evaluate the status of the patient's Alzheimer's Disease.

Sub D2
3. (amended) The method [for diagnosing, subtyping or monitoring Alzheimer's disease according to] of claims 1 or 2, wherein [the assay is] the assaying step comprises performing an assay selected from the group consisting of a radioimmunoassay, an enzyme-linked immunosorbant assay, a sandwich assay, a precipitin reaction, a gel immunodiffusion assay, an agglutination assay, a fluorescent immunoassay, a protein A immunoassay [or] and an immunoelectrophoresis assay.

C2
5. (amended) A kit for detecting the presence of an anti-tau-peptide autoantibody in a sample of [body fluid] blood or cerebrospinal fluid comprising a tau-peptide and means for detecting the binding of the autoantibody with the tau-peptide.

Please enter the following new claims:

6. The method of claim 1 wherein the sample is a sample of blood.

C3
7. The method of claim 3 wherein the sample is a sample of blood.--

REMARKS

Claims 1-3 and 5-7 are in the case.

Claims 1-3 have been amended to remove the words "subtyping and monitoring" from the preamble. Claims 1, 2 and 5 have been amended to replace "a sample of body fluid" by --a sample of blood or cerebrospinal fluid--. Support for the amendment is found at page 1, line 9 and page 26, lines 14-19. Claims 1 and 2 have been amended to recite a comparison step and to replace "the presence" with "a concentration". Support for the amendment may be found at page 27, lines 12-14.

Claim 1 has been amended to delete the phrase "derived from proteolytic action on neurofibrillary tangles resulting in fragments." Support for this amendment is found in the paragraph bridging pages 12-13.

Claim 3 has been amended to replace "the assay is" with --the assaying step comprises performing-- to conform to claiming formalities.

New Claim 6 has been added that specifically claims the method of Claim 1 wherein the sample is a sample of blood. Support for the new claim is found at page 1, line 9 and page 26, lines 14-19. Claim 7 is drawn to the method of Claim 6 performed by the particular assays recited in Claim 3, as filed. Support for Claims 7 can be found as for Claims 6 and in Claim 3 as filed.

I. THE APPLICATION ENABLES AN OPERABLE INVENTION HAVING A PRACTICAL UTILITY AND THE CLAIMS CONFORM TO 35 U.S.C § 112

Pending Claims 1-3 and 5 were rejected under 35 U.S.C. § 101 and the specification objected to under 35 U.S.C. § 112 1st ¶ because the invention is alleged to be inoperable.

The Applicant respectfully traverses. Submitted herewith are Declarations Under Rule 132 of the Inventor, Dr. H. Paul Voorheis and of Dr. Davis Coakley of the Medical School of St. James Hospital, Dublin Ireland, showing that an assay performed according to the teaching of the specification clearly distinguishes between a population of 18 individuals having a diagnosis of Alzheimer's Disease and 18 controls.

Dr. Voorheis, who is a trained physician and former medical practitioner states in ¶ 8 of his Declaration Under Rule 132:

The results of the assay were closely correlated with the clinical diagnosis of Alzheimer's Disease. Indeed, considering that clinical and pathological diagnoses of Alzheimer's Disease are not infrequently discordant, the correlation . . . is as close as could be expected. Thus, the assay performed according to the teaching of the application was operative, in this trial, to diagnose Alzheimer's Disease with high specificity and sensitivity.

To the best of the Applicant's knowledge, these data represent the first example of a diagnostic test for Alzheimer's Disease that is both objective and can be performed without intrusive entry into the Central Nervous System of the subject. Previous diagnostic methods required, at least, lumbar puncture to obtain a sample of cerebrospinal fluid. See "Laboratory Diagnostic Tests for Alzheimer's Disease" by Iqbal, K., et al., in ALZHEIMER'S DISEASE AND RELATED DISORDERS (Alan Liss, Inc., 1989) pg 679-687. (Ref. AE in the Information Disclosure submitted herewith)

The Office Action alleges that the teaching of the application is insufficient to practice to the invention without undue experimentation because the application does not teach the absolute level of τ -peptide, or its equivalent, that is diagnostic of Alzheimer's Disease. Applicant respectfully traverses on the grounds that the determination of the diagnostic level is well within the level of ordinary skill in the art. As the data presented herewith reveals the variation in the apparent levels of τ -peptide in normal human plasma is small compared to the difference between the levels of normal subjects and Alzheimer's patients. Therefore, the precise level which is considered diagnostic may be set within a wide range without effecting the validity of the test. Such a determination is submitted to be well within the capacities of one skilled in the field.

Further, any particular diagnostic level would be dependent upon the specificity and variability of the particular assay that the practitioner employs. Therefore, the specification of such a diagnostic level for one assay is not, generally, transferable to other assays without further, albeit routine, investigation.

The Office Action further alleges that the invention is inoperable because the application does not teach how to assay for τ -peptide fragments "derived from proteolytic action on neurofibrillary tangles" and because the application does not teach a means of distinguishing between τ -peptide fragments and τ -proteins.

In response to the first allegation and to more particularly point out and precisely claim the invention, the Applicant has amended the claim to delete the postulated origin

of the τ -peptide fragments. In response to the second allegation the Applicant respectfully points out that τ -protein is not normally in the blood or in cerebrospinal fluid. Beginning at page 13, line 3, the specification points out: "The detection or quantitative measurement of any of these τ -proteins or fragments thereof in body fluids in accordance with this invention is useful in confirming a clinical diagnosis of Alzheimer's Disease" (emphasis added).

Thus, the operability of the invention does not require that the distinction of concern to the Examiner be enabled by the specification. The claimed invention recites that the concentration of τ -peptides be assayed; the application teaches that there is no technical requirement that the assay means distinguish between the peptide of interest and its precursor protein.

Claims 1-3 were rejected under 35 U.S.C. § 112, 2nd ¶. The claims as amended obviate the rejection. The preambles to Claims 1-3, as amended, recite that the claimed invention is a method of diagnosing Alzheimer's Disease. Claim 3 has been amended to replace "wherein the assay is" with the phrase "wherein the assaying step comprises."

II. THE REJECTIONS OVER *WOLOZIN* AND *KSIEZAK-REDDING* ARE IMPROPER AND SHOULD BE WITHDRAWN

Claims 1 and 3 were rejected over *Wolozin et al.*, Ann. Neurol. 22:521-26 (*Wolozin*) in view of *Ksiezak-Redding et al.*, 1990, J. Neuroscience Research 25:412-19, (*Ksiezak-Redding*) based on 35 U.S.C. § 102(b) or alternatively on 35 U.S.C. § 103.

Wolozin is alleged to have identified an monoclonal antibody ("Alz 50") that reacts with a protein ("A68") which is present in the cerebrospinal fluid of Alzheimer's patients but not others. *Ksiezak-Redding* is alleged to have shown that the monoclonal antibody Alz 50 reacts with an N-terminal peptide of the τ -protein.

Applicant traverses. A careful reading of *Ksiezak-Redding* shows that the epitope recognized by Alz 50 was not shown to be absent from a peptide containing the N-terminal 200 residues of the τ -protein. At page 413, first full paragraph, *Ksiezak-Redding* states:

Lack of reactivity of the [λ gt11/ τ -protein] fusion protein with Alz 50 prompted the suggestion that the epitope was wither conformationally or post-translationally modified. Therefore, to study the seemingly conformationally dependent epitopes such as Alz 50 . . . we digested τ with chymotrypsin or trypsin and immunoblotted the fragments with Alz 50.

At page 416, first full paragraph, *Ksiezak-Redding* states:

The immunoblots of Alz 50 and Tau 60 were similar to each other, but different from those of other antibodies by the lack of staining of fragments smaller than 38 kDa.

Lastly, at page 417, the first paragraph of the discussion of *Ksiezak-Redding* states:

This study showed that the Alz 50-reactive epitope is sensitive to protease digestion. After the chymotrypsin cleavage of a 3-4 kDa fragment from the parent τ polypeptides and their endogenous fragments, the Alz 50 epitope was no longer detected.

Applicant submits that a fair reading of *Ksiezak-Redding* leads to the conclusion that, contrary to the allegations of the Examiner, Alz 50 recognizes only the intact τ -protein and does not recognize an isolated N-terminal fragment. The Office Action fails to recognize the distinction between the portion of a protein molecule that is necessary for binding of an antibody and the binding of the antibody to an isolated peptide. The Alz 50 epitope was reported to be localized to the N-terminal, i.e., the presence of the N-terminal fragment is necessary for binding. However, *Ksiezak-Redding* was equally definite in stating that no binding to a fragment of molecular weight less than 38kD was observed. Given the average molecular weight of a peptide is 110 Da/residue¹, it follows that the smallest fragment that *Ksiezak-Redding* identifies as having a Alz 50 epitope is about 340 amino acids.

Applicant further requests the Examiner's attention to the fact that although *Wolozin* was able to detect A68 in CSF only after concentrating the CSF by 87.5 fold (3.5 ml into 40 μ L) and the only method disclosed was a Western blot, indicating that very low levels of A68 are present. Applicant respectfully points out that Claim 3, as amended, expressly recites the assay methods to be employed to determine the concentration of τ -peptide and does not include the Western blot method of *Wolozin*.

The Office Action further alleges, in the alternative, that Claims 1 and 3 are obvious over the combination of *Ksiezak-Redding* and *Wolozin*. *Wolozin* is alleged to teach that there is detectable amounts of A68 in the cerebrospinal fluid of Alzheimer's suffers. *Ksiezak-Redding* is alleged to teach that A68 is a species of τ -protein and to disclose monoclonal antibodies reactive with N-terminal peptides thereof. It is alleged

¹ Stryer, Lubert, BIOCHEMISTRY, (W.H. Freeman & Co., New York) p 22.

that one of ordinary skill utilizing the antibodies of *Ksiezak-Redding* to detect the A68 protein would have had a reasonable expectation of obtaining a method to diagnose Alzheimer's Disease. Applicant respectfully traverses.

Wolozin discloses the presence of an abnormal protein in the cerebrospinal fluid (CSF) of Alzheimer's patients. This protein is present in the brain tissue of such patients and not in that of normals. *Wolozin* does not suggest, much less teach, whether the levels of A68 together with its non-pathological homologs are elevated or even altered in Alzheimer's patients. Thus, one of ordinary skill in the art using the antibodies of *Ksiezak-Redding*, which do not differentiate between pathologic and normal proteins, would have had no reasonable expectation that the CSF levels of normal τ -proteins or the level of the total of normal τ -proteins and A68 would be elevated or that an operable diagnostic method could be so obtained.

Further, Applicant respectfully points out that the newly submitted Claim 6 is directed towards a diagnostic method wherein a sample of blood is assayed for the presence of τ -peptides. *Wolozin* is completely deficient in suggestion that A68 or any other protein is present in the blood of Alzheimer's patients.

The rejection of Claim 4 over Kosik et al., 1988, NEURON 1:817-25 is moot in view of the cancellation of Claim 4 without prejudice.

III. THE REJECTION OVER *GASKIN* AND OTHERS IS IMPROPER AND SHOULD BE WITHDRAWN

The Office Action indicates that claims 2, 3 and 5 are rejected over the combination of Gaskin, F., et al., 1986, CLINICAL RESEARCH 34(2):669A (Abstract) (*Gaskin*) and Grundke-Iqbal, I. et al., 1986, J.BIOL.CHEM. 261:6084 (*Iqbal*) in view of Singh et al., IMMUNOL. LETT. 12:277 and Foley et al., 1988, J. NEUROL. 235:466-71 (the "secondary references").

Gaskin is alleged to have taught that autoantibodies to Neurofibrillary Tangles (NFT) are common in Alzheimer's patients, but admittedly *Gaskin* does not further identify the antigen recognized by the anti-NFT autoantibodies. *Iqbal* is alleged to have taught that τ -proteins are a component of NFT. The secondary references are alleged to provide further motivation to make the combination of *Gaskin* and *Iqbal* by teaching that Alzheimer's Disease is associated with autoantibodies directed toward multiple antigens.

The Examiner asserts that one of ordinary skill combining *Gaskin* and *Iqbal* would conclude that the autoantibodies were directed towards τ -proteins.

Applicant respectfully traverses. "The test for obviousness is what the combined teachings of the [prior art] references would have suggested to one of ordinary skill in the art" *In re Young*, 18 USPQ2d 1089, 1091 (Fed. Cir. 1991) (following *In re Keller*, 208 USPQ 871, 881 (CCPA 1981)). Applicant brings to the Examiner's attention the fact that F. Gaskin has continued to investigate the specificity of the anti-NFT autoantibodies disclosed in her abstract. These studies specifically investigated whether the autoantibodies were reactive with phosphorylated τ -proteins. Her conclusion was that none of the autoantibodies reactive with NFT in Alzheimer's Disease brain reacted with τ -proteins. Kingsley, B.S., Gaskin, F., et alia, 1988, J. NEUROIMMUNOLOGY 19:89-99, 97 (*Gaskin II*) (Ref. AC in the Information Disclosure submitted herewith). Thus, the teaching of the prior art as a whole suggests that the anti-NFT autoantibodies, first reported in *Gaskin*, are not directed to τ -proteins.

Applicant respectfully request that the rejection of Claims 2, 3 and 5 be reconsidered and withdrawn in view of *Gaskin II* which teaches that the autoantibodies disclosed in *Gaskin* are not directed toward τ -proteins.

IV. CONCLUSION

Applicant respectfully submits that the rejections of the Office Action of May 5, 1994 are either obviated or overcome by the Declarations of Drs. Davis Coakley and H. Paul Voorheis, the inventor, and the foregoing amendments. Applicant accordingly requests the speedy allowance of all pending claims.

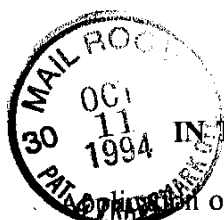
Respectfully submitted,

S. Leslie Misrock

Date 5 October 1994

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Enclosure



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor of: Voorheis, H. Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: Bidwell, C.

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

RECEIVED

OCT 21 1994

DECLARATION OF DAVIS COAKLEY, M.D., UNDER 37 C.F.R. § 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

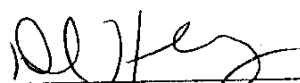
I, DAVIS COAKLEY DECLARE AS FOLLOWS:

1. I am a citizen of the Republic of Ireland residing at 57 Mount Anville, Dublin 14, Ireland.
2. I received M.B., B.Ch., B.A.O. (the equivalent to an American M.D.) degree in 1971 and an M.D. degree in 1978 from the National University of Ireland. I have been, since 1991, President of the Irish Gerontologic Society; since 1984, a Fellow of the Royal College of Physicians in Ireland; and, since 1994, a Fellow of the Royal College of Physicians (London). A copy of my *curriculum vitae* is attached hereto as Exhibit A.
3. My medical practice is conducted at St. James Hospital, Dublin. I have been, since 1979, a Consultant Physician in geriatric medicine, St. James Hospital, Dublin, Ireland and, since 1988, the Director of Mercer's Institute for Research on Ageing at the same institution. I am an Associate Professor of Geriatric Medicine at Trinity College, Dublin, Ireland and Dean of Health Sciences at Trinity College.

CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 5, 1994.

Daniel Hansburg
(Name)


(Signature)

36,156

(Reg. No.)

October 5, 1994
(Date of Signature)

PENY2-305745.1

PROFESSOR DAVIS COAKLEY

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M.B., B.Ch., B.A.O. 1971
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M.D. (N.U.I.) 1978
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F.R.C.P. (LOND) 1994

POSITIONS:

Consultant Physician in Geriatric Medicine, St. Jame's Hospital, Dublin (October 1979 to date)

Director of Post Graduate Medical Education, Trinity College, Dublin (1984 - 1990)

Director, Mercer's Institute for Research on Ageing, St. Jame's Hospital (1988 to date).

Associate Professor of Geriatric Medicine, Trinity College, Dublin (1986 to date)

Dun's Librarian, Royal College of Physicians of Ireland (1991 to date)

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PREVIOUS POSITIONS:

Senior Registrar, University Hospital of Wales, Cardiff

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SOCIETIES:

British Geriatric Society

Irish Gerontological Society
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President (1991 to date)

Irish Society of Physicians in Geriatric Medicine

Irish Neurological Association

Royal Academy of Medicine in Ireland

Stroke Association of Ireland
Founder member and former Chairman

Irish Association of Rehabilitation Medicine

PENY2-308465.1

SELECTED RELEVANT &
MEDICAL AND SCIENTIFIC
PUBLICATIONS:

Books:

Lectures in Geriatric Medicine (Editor)
University Hospital of Wales (1978)

Acute Geriatric Medicine (Editor)
Croom Helm, London and PSG Littleton
Mass, U.S.A. (1981)

Establishing a Geriatric Service (Editor)
Croom Helm, London and Canberra (1982)

Nursing and the Doctor (Co-editor with
V. Hanson)
Pitman Medical London (1982)

Minute Eye Movement and Brain Stem Function
(Author). CRC Press, Florida, U.S.A. (1983)

Physiotherapy and the Elderly Patient
(Co-author with P. Wagstaff)
Croom Helm, London and Canberra
Aspen Publishers, Maryland, U.S.A. (1988)

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Exhibit B

views & reviews

Article abstract—Clinical criteria for the diagnosis of Alzheimer's disease include insidious onset and progressive impairment of memory and other cognitive functions. There are no motor, sensory, or coordination deficits early in the disease. The diagnosis cannot be determined by laboratory tests. These tests are important primarily in identifying other possible causes of dementia that must be excluded before the diagnosis of Alzheimer's disease may be made with confidence. Neuropsychological tests provide confirmatory evidence of the diagnosis of dementia and help to assess the course and response to therapy. The criteria proposed are intended to serve as a guide for the diagnosis of probable, possible, and definite Alzheimer's disease; these criteria will be revised as more definitive information becomes available.

Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease

Guy McKhann, MD; David Drachman, MD; Marshall Folstein, MD; Robert Katzman, MD;
Donald Price, MD; and Emanuel M. Stadlan, MD

Alzheimer's disease is a brain disorder characterized by a progressive dementia that occurs in middle or late life. The pathologic characteristics are degeneration of specific nerve cells, presence of neuritic plaques, and neurofibrillary tangles. Alterations in transmitter-specific markers include forebrain cholinergic systems, and, in some cases, noradrenergic and somatostatinergic systems that innervate the telencephalon.

A Work Group on the Diagnosis of Alzheimer's Disease was established by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA). The group intended to establish and to describe clinical criteria for the diagnosis of Alzheimer's disease of particular importance for research protocols and to describe approaches that would be useful for assessing the natural history of the disease. The need to refine clinical diagnostic criteria has been emphasized because 20% or more of cases with the clinical diagnosis of Alzheimer's disease are found at autopsy to have other conditions and not Alzheimer's disease. Moreover, therapeutic trials can be meaningfully compared only if uniform criteria are used for diagnosis and response to treatment.

The need for this report was suggested by the National Advisory Council of the NINCDS. The

report has been reviewed by workshop participants, representatives of the National Advisory Neurological and Communicative Disorders and Stroke Council, representatives of the ADRDA, and designated reviewers representing professional societies concerned with the diagnosis of Alzheimer's disease. (For list of professional societies and designated reviewers, see page 943.)

The report was developed by subgroups that addressed medical history, clinical examination, neuropsychological testing, and laboratory assessments; the report was then discussed in plenary session. Based on a consensus of the participants, criteria were developed to serve as a clinical basis for diagnosis. These criteria should be useful also for comparative studies of patients in different kinds of investigations, including case control studies, therapeutic trials, evaluation of new diagnostic laboratory tests, and clinicopathologic correlations.

The criteria are not yet fully operational because of insufficient knowledge about the disease. The criteria are compatible with definitions in the current Diagnostic and Statistical Manual of Mental Disorders (DSM III) and in the International Classification of Diseases. These criteria must be regarded as tentative and subject to change. Additional longitudinal studies, confirmed by autopsy, are necessary to establish the validity of these criteria in com-

*For Work Group Participants and Affiliations, see page 943.

Accepted for publication March 20, 1984.

Address correspondence and reprint requests to Dr. Stadlan, 7550 Wisconsin Avenue, Federal Building, Room 700, Bethesda, MD 20205.

Table 1. Criteria for clinical diagnosis of Alzheimer's disease

I. The criteria for the clinical diagnosis of PROBABLE Alzheimer's disease include:

dementia established by clinical examination and documented by the Mini-Mental Test,¹ Blessed Dementia Scale,² or some similar examination, and confirmed by neuropsychological tests;

deficits in two or more areas of cognition;

progressive worsening of memory and other cognitive functions;

no disturbance of consciousness;

onset between ages 40 and 90, most often after age 65; and

absence of systemic disorders or other brain diseases that in and of themselves could account for the progressive deficits in memory and cognition

II. The diagnosis of PROBABLE Alzheimer's disease is supported by:

progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia), and perception (agnosia);

impaired activities of daily living and altered patterns of behavior;

family history of similar disorders, particularly if confirmed neuropathologically; and

laboratory results of:

normal lumbar puncture as evaluated by standard techniques,

normal pattern or nonspecific changes in EEG, such as increased slow-wave activity, and

evidence of cerebral atrophy on CT with progression documented by serial observation.

III. Other clinical features consistent with the diagnosis of PROBABLE Alzheimer's disease, after exclusion of causes of dementia other than Alzheimer's disease, include:

plateaus in the course of progression of the illness;

associated symptoms of depression, insomnia, incontinence, delusions, illusions, hallucinations, catastrophic verbal, emotional, or physical outbursts, sexual disorders, and weight loss;

other neurologic abnormalities in some patients, especially with more advanced disease and including motor signs such as increased muscle tone, myoclonus, or gait disorder;

seizures in advanced disease; and

CT normal for age.

IV. Features that make the diagnosis of PROBABLE Alzheimer's disease uncertain or unlikely include:

sudden, apoplectic onset;

focal neurologic findings such as hemiparesis, sensory loss, visual field deficits, and incoordination early in the course of the illness; and

seizures or gait disturbances at the onset or very early in the course of the illness.

V. Clinical diagnosis of POSSIBLE Alzheimer's disease:

may be made on the basis of the dementia syndrome, in the absence of other neurologic, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in the onset, in the presentation, or in the clinical course;

may be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of the dementia; and

should be used in research studies when a single, gradually progressive severe cognitive deficit is identified in the absence of other identifiable cause.

VI. Criteria for diagnosis of DEFINITE Alzheimer's disease are:

the clinical criteria for probable Alzheimer's disease and

histopathologic evidence obtained from a biopsy or autopsy.

VII. Classification of Alzheimer's disease for research purposes should specify features that may differentiate subtypes of the disorder, such as:

familial occurrence;

onset before age of 65;

presence of trisomy-21; and

coexistence of other relevant conditions such as Parkinson's disease.

parison with other criteria such as DSM III.

Criteria for dementia syndrome. Dementia is the decline of memory and other cognitive functions in comparison with the patient's previous level of function as determined by a history of decline in performance and by abnormalities noted from clinical examination and neuropsychological tests. A diagnosis of dementia cannot be made when consciousness is impaired by delirium, drowsiness, stupor, or coma or when other clinical abnormalities prevent adequate evaluation of mental status. Dementia is a diagnosis based on behavior and cannot be determined by computerized tomography, electroencephalography, or other laboratory instru-

ments, although specific causes of dementia may be identified by these means.

Criteria for Alzheimer's disease. Alzheimer's disease is a progressive, dementing disorder, usually of middle or late life. The clinical criteria for the diagnosis of PROBABLE, POSSIBLE, and DEFINITE Alzheimer's disease are outlined in table 1. A clinical diagnosis of probable Alzheimer's disease can be made with confidence if there is a typical insidious onset of dementia with progression and if there are no other systemic or brain diseases that could account for the progressive memory and other cognitive deficits. Among the disorders that must be excluded are manic-depressive disorder, Parkinson's

disease, multi-infarct dementia, and drug intoxication; less commonly encountered disorders that may cause dementia include thyroid disease, pernicious anemia, luetic brain disease and other chronic infections of the nervous system, subdural hematoma, occult hydrocephalus, Huntington's disease, Creutzfeldt-Jakob disease, and brain tumors.

A diagnosis of definite Alzheimer's disease requires histopathologic confirmation. A clinical diagnosis of possible Alzheimer's disease may be made in the presence of other significant diseases, particularly if, on clinical judgment, Alzheimer's disease is considered the more likely cause of the progressive dementia. The clinical diagnosis of possible rather than probable Alzheimer's disease may be used if the presentation or course is somewhat aberrant. The information needed to apply these criteria is obtained by standard methods of examination: the medical history; neurologic, psychiatric, and clinical examinations; neuropsychological tests; and laboratory studies.

Medical history. A medical history should be taken from the patient and from an informant who is well acquainted with the affected individual. This approach is essential to establish a history of progressive deterioration and for identifying tasks that the patient can no longer perform adequately. A diary maintained by an observer may be very helpful in documenting changes in various functions. The history discloses abnormalities including impaired memory and other cognitive functions, impaired activities of daily living, alterations in mood, often delusions and illusions, and sometimes hallucinations. Common complaints of patients or families include forgetfulness about appointments or errands; inability to find the way to an accustomed destination; inability to use money and instruments of daily living such as a telephone; deterioration in work or homemaking performance; difficulty adapting to changes in the workplace; difficulties in dressing, reading, and writing; and inability to recognize previously familiar individuals.

Clinical examination. The clinical examination provides data to fulfill inclusionary and exclusionary criteria for the diagnosis of Alzheimer's disease and to document symptoms such as delusions or depression that identify subgroups of patients important both for research studies and for patient care. Mental status testing, an essential component of the clinical examination, includes specific assessment of orientation, registration, attention, calculation, recent recall, naming, repeating, understanding, reading, writing, and ability to draw or copy. Because cognitive impairment may occur in depressive syndromes, it is important to inquire about affective state and depressive symptoms, such as disturbed sleep and weight loss, before diagnosing Alzheimer's disease. Inquiry specifically about the presence of delusions and hallucinations is needed to identify subgroups. Both symptoms may be experienced in a

variety of neuropsychiatric disorders, which may or may not have known organic substrates.

Quantitative aids to the clinical examination include the Mini-Mental State Examination¹ for cognitive screening; the Blessed Dementia Scale² for clinical symptoms and social function; the Hamilton Depression Scale³ for severity of depression; the Present State Examination⁴ for anxiety, depression, delusions, and hallucinations; and the Hachinski Scale^{5,6} for estimating the likelihood of multi-infarct dementia. A complete psychiatric evaluation is needed to exclude the various psychiatric disorders.

Complete examination of sensory and motor systems (including cranial nerves, tone, reflexes, coordination, gait, and proprioception) is needed to exclude other neurologic disorders. In early stages, patients are alert and free of other neurologic changes related to the dementia except for the occasional presence of snout reflex, jaw jerk, rigidity, or myoclonus, all of which may be encountered in nondemented elderly people. As the disease progresses, some patients become apathetic or show irritability, agitation, paranoid ideas, sleep disorders, or incontinence. In the very advanced stages, patients may become mute and lose all ability to communicate.

Neuropsychological testing. Neuropsychological tests may provide additional information for the diagnosis of dementia. Because there are no normative population standards for many of these tests, abnormal performance can be determined only by comparison with a normal control group matched for age, sex, and local education. A score falling in the lowest fifth percentile of an individual's normal control group may be designated as "abnormal." One or more abnormal scores will identify an individual for research purposes who is highly likely to be cognitively impaired. Progressive worsening can be established by comparison with the patient's previous performance on these tests. Although there is continued debate about the tests that best measure these functions, the Work Group did make some suggestions (table 2).

Similar series of tests can be used to assess less severely affected patients by increasing the complexity of the neuropsychological tests. Further modification in the test procedure may be needed to detect impairment in highly intelligent patients. Confirmation of the dementia syndrome by neuropsychological tests should be based on measurable abnormalities in two or more aspects of cognition.

In longitudinal assessment, many patients with Alzheimer's disease show progressive loss of recent memory followed by disorders of language, praxis, or visual perception. In some patients with Alzheimer's disease, however, the first symptoms are difficulty in finding words, impaired visual perception, or apraxia, with memory impairment and other symptoms and signs appearing later.

Although neuropsychological tests are presently used primarily to provide confirmatory evidence for

Table 2. Neuropsychological evaluation

The major cognitive processes that are impaired in Alzheimer's disease, with examples of the kinds of tests used to assess these functions, include:

orientation to place and time, graded by a test such as the Mini-Mental State Examination¹;

memory evaluated by tests such as a free-recall test⁷ of concrete nouns, a 3-4 paired-associate learning test (verbal and nonverbal) by use of a recognition paradigm, the Recognition Span Test,⁸ and the Brown-Peterson Distractor Test^{9,10} (stopping the task when the patient fails or begins to produce the distractor instead of the stimulus trigrams);

language skills tested by examination of verbal fluency of the semantic or category type, with the examiner writing responses, and by other tests such as the Boston Naming Test¹¹ (preferably one of the abbreviated forms), the Boston Diagnostic Aphasia Examination,¹² the Western Aphasia Test,¹³ and the Token Test¹⁴⁻¹⁶ with Reporter's Test¹⁷;

praxis evaluated by tests¹⁸ such as those in which the patient copies a drawing (cube, daisy, clock, or house) or performs the block design subtest of the Wechsler Adult Intelligence Scale¹⁹;

attention monitored by tests such as a reaction-time task²⁰ or by the Continuous-Performance Test²¹;

visual perception studied by use of a variety of tasks, such as the Gollin Incomplete-Pictures Test²² and the Hooper Test²³;

problem-solving skills determined by tests such as the Wisconsin Card Sorting Test^{24,25} or The Poisoned Food Problem Task of Arenberg^{26,27}; and

social function, activities of daily living,^{28,29} and instrumental activity of daily living,³⁰ assessed by methods similar to those described in the Philadelphia Geriatrics Center Forms.¹¹

the diagnosis of dementia, these tests are valuable for determining patterns of impairment, for assessing changes in impairment over time and after drug treatment or rehabilitation, and for establishing correlations of abnormal performance with laboratory and neuropathologic examinations.

Laboratory assessments. Clinical assessment and neuropsychological tests provide information to meet the criteria for clinically probable Alzheimer's disease. At present, there are no specific diagnostic laboratory tests for Alzheimer's disease, but some tests can enhance diagnostic accuracy by identifying other causes of the dementia syndrome. Moreover, as suggested by the Work Group, the laboratory approaches described below used quantitatively in longitudinal studies should help to clarify the natural history of Alzheimer's disease, possibly provide information needed in subtyping the disease, and permit measurement of efficacy of therapeutic interventions. Some of these techniques, particularly positron emission tomography, are strictly investigative tools and not readily available outside of research institutions.

Electrophysiologic methods. The EEG of some

patients with Alzheimer's disease shows increased slow-wave activity that may become more pronounced with progression of the disease. Evoked potentials (EP) are brain waves associated with sensory or other events that may be auditory, somatosensory, or visual. Endogenous or cognitive potentials, such as P300, are thought to reflect speed of cognition. The latency of P300 is altered with age, and there appears to be an increased latency of P300 potentials in 50 to 80% of patients with Alzheimer's disease compared with age-matched control subjects. These changes occur in different dementias and are not specific to Alzheimer's disease. The P300 wave, however, is normal in depressive syndromes and may therefore be useful in differentiating the dementia of Alzheimer's disease from the dementia of depressive syndromes, particularly when adequate normal data become available.

Computerized tomography. CT is useful in the diagnosis of Alzheimer's disease because it permits the exclusion of other disorders such as subdural hematoma, brain tumor, hydrocephalus, and dementia associated with vascular disease. The technique can delineate gyri and sulci and quantitate tissue densities, ventricular size, CSF volume, and brain mass. In Alzheimer's disease, the volume of the ventricular system and the width of the third ventricle are increased, gyri are narrowed, and sulci are widened; however, these general patterns may not be particularly useful as diagnostic criteria in individual cases. Furthermore, available data do not indicate how well a qualitative observation correlates with the magnitude of cognitive abnormality or with evidence of progression of disease. There is a pressing need for quantitative CT studies of Alzheimer's disease patients during the course of disease and for correlation of CT images with clinical signs, neuropsychological test results, and autopsy findings.

Regional cerebral blood flow. Measurement of regional cerebral blood flow (rCBF), including ¹³³xenon clearance, may help differentiate Alzheimer's disease and dementia associated with cerebrovascular disease. In multi-infarct dementia (MID), early changes include decreased autoregulation; in the later stages of MID, rCBF and oxygen consumption are decreased. In patients who have Alzheimer's disease, rCBF and cerebral metabolic rate are decreased; but A-V differences, carbon dioxide responses, and autoregulation are preserved.

Positron emission tomography. Positron emission tomography (PET) is a research technique that allows quantitative assessment of the rate of glucose utilization, oxygen consumption, and rCBF. With some isotopes, these characteristics can be assessed during neuropsychological testing; moreover, ¹¹C-markers may permit the use of retest paradigms. Early reports suggest that rCBF determined by PET may be reduced in areas of encephalomalacia. In contrast, most patients with Alzheimer's disease show cerebral hypometabolism when compared with

age-matched controls. These changes correlate with disease severity and may be correlated with neuropsychological test performance. For example, speech impairment may be correlated with decreased activity in the left hemisphere, whereas impaired performance on spatial tasks may be more closely correlated with impaired activity in the right hemisphere. Different approaches may be necessary for delineating presynaptic and postsynaptic markers of transmitter systems, as recently achieved with PET images of the dopamine system. Since PET reveals a significant variation even among normal subjects, any changes may have to be severe to be detected. The value of PET studies in determining the stage of disease, in documenting progression, and in assessing the effects of treatment is unknown.

Magnetic resonance imaging. The proton nuclear magnetic resonance (NMR) image, or magnetic resonance imaging (MRI), reveals the demarcation of gray and white matter of the brain and has therefore proved useful in studies of demyelinating disorders. Although the method has not been applied systematically to the study of dementia, it has potential for differentiating between Alzheimer's disease, multi-infarct dementia, and low-pressure hydrocephalus. Information should soon be available about the usefulness of MRI in the diagnosis of Alzheimer's disease.

Examination of body fluids and nonneural tissues. In the diagnosis of Alzheimer's disease, studies of blood and CSF are helpful in excluding chronic infections, such as cryptococcal meningitis and syphilis, and other disorders. To date, definitive diagnostic information about Alzheimer's disease from blood or CSF has not been sought consistently, but CSF should be studied to demonstrate neurotransmitters, metabolites, and synthesizing and degradative enzymes. Other techniques, such as sophisticated radioimmunoassays with specific antibodies, may be useful for detecting markers of the disease, such as constituents associated with the development of neurofibrillary tangles and neuritic plaques. Specific abnormalities have not been detected in nonneural tissues.

Acknowledgments

The authors express their appreciation to Dr. George Eaves, NINCDS, for editorial and stylistic contributions, to Dr. Jason Brandt, Johns Hopkins University, and Dr. Asenath LaRue, UCLA, for verifying the accuracy of the bibliographic references, and to Ms. Paula J. Kirby, NINCDS, for patient and excellent secretarial services.

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 31. Lawton MP, Moss M, Fulcomer M, Kleban MH. A research and service-oriented multilevel assessment instrument. *J Gerontol* 1982;37:91-9.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
VOORHEIS, H. Paul

Serial No.: 08/159,969

Filed: November 30, 1993

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Group Art Unit: 1802

Examiner: C. Bidwell

Attorney Docket No.: 4697-059

DECLARATION OF H. PAUL VOORHEIS, M.D., PH.D.,
SUBMITTED UNDER RULE 132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

SIR:

I, HOWARD PAUL VOORHEIS, declare and state as follows:

1. I am a citizen of the United States, a domiciliary of the United Kingdom and have a residence at 37 Upper Mount Pleasant Avenue, Rathmines, Dublin 6, Ireland.
2. I am the inventor of the invention described in patent application Serial No. 08/159,969, filed on November 30, 1993 entitled "DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE." I am an employee of Trinity College, Dublin 2, Ireland, the assignee of the above-identified patent application.
3. I have received an M.D. degree, 1965, from the University of Michigan and a Ph.D. degree, 1975, in Biochemistry from the University of Cambridge. From 1979 to present I have been a Senior Lecturer in Biochemistry at Trinity College, Dublin,

CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 5, 1994.

Daniel Haneburg
(Name)

[Signature]
(Signature)

26,156

(Reg. No.)

October 5, 1994
(Date of Signature)

PENY2-307770.1

Ireland. I was licensed to practice medicine by the state of Michigan in 1965. My qualifications and training are acceptable for registration as a physician licensed to practice by the General Medical Council of Great Britain. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

4. Following the teaching of the specification of the above-identified application at page 27, line 31 - page 28, line 27, I conducted a forward double sandwich assay to detect peptides of τ -protein in samples of serum of 18 patients diagnosed as having Alzheimer's Disease by Dr. Davis Coakley and his colleagues and of 18 age and sex matched controls.

5. The technical details of experimental procedure are provided in Exhibit B, attached hereto. All reagents were purchased from commercial scientific suppliers. Among the reagents used was the monoclonal antibody, "Tau 1", a monoclonal antibody raised by Dr. L. Binder of the University of Alabama, to bovine microtubule associated proteins the specificity of which is described by Kosik, K.S., et al., 1988, NEURON 1:817-825, which reference, I understand, is already of record in the prosecution of the above-identified application. Tau 1 is commercially available from the Boehringer-Mannheim Company as catalog No. 1289-977.

6. The results of these studies are presented in tabular and graphical form in Exhibit C, attached hereto. The results are reported as the optical density (O.D.) due to the conversion of a chromophore precursor by an enzyme-reporter molecule coupled to the third step antibody in the double sandwich assay. The mean O.D. ($\pm$ std. dev.) of the 18 control sample was 0.108 ± 0.02 . The eighteen (18) patient samples were divided into three (3) groups of six (6) each depending upon the clinical assessment of the severity of the subject's disease. The results of these groups were (mean $\pm$ std. dev.): mild, 0.517 ± 0.244 ; moderate, 0.883 ± 0.335 ; severe, 0.799 ± 0.510 . Of the eighteen Alzheimer's patients, two were found to have O.D. levels of 0.09 and 0.104, which levels were within the normal range, (0.075 - 0.134). These subjects may well not have had Alzheimer's Disease. Approximately 20% of clinically diagnosed "Alzheimer's Disease" patients who come to *post mortem* are found to have had other pathological entities. The third lowest sample had an O.D. of 0.24. This value was greater than the

upper limit of the normal samples, 0.134 by a margin of more than five (5) times the standard deviation of the normal population (0.201).

7. These results show that an assay performed according to the teaching of the specification divides the clinical material into two distinct populations. All 16 samples found to be in the "high O.D." population were clinically diagnosed as having Alzheimer's Disease. By contrast, 18 of the 20 samples in the low population were not diagnosed as having Alzheimer's Disease.

8. The results of the assay were closely correlated with the clinical diagnosis of Alzheimer's Disease. Indeed, considering that clinical and pathological diagnoses of Alzheimer's Disease are not infrequently discordant, the correlation with the clinical is as close as could be expected. Thus, the assay performed according to the teaching of the application was operative, in this trial, to diagnose Alzheimer's Disease with high specificity and sensitivity. The assay, if generally available, would be of practical use in the practice of medicine.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and any patent issuing thereon.

Date: 5 October 1994

H. Paul Voorheis
H. PAUL VOORHEIS, M.D., Ph.D.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
VOORHEIS, H. Paul

Serial No.: 08/159,969

Filed: November 30, 1993

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

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Group Art Unit: 1802

Examiner: C. Bidwell

Attorney Docket No.: 4697-059

DECLARATION OF H. PAUL VOORHEIS, M.D., PH.D.,
SUBMITTED UNDER RULE 132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

COPY

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CERTIFICATE UNDER 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on October 5, 1994.

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PENY2-307770.1

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- 13) 'Studies on the Phosphoketolase Pathway,
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- 14) IgM-Dependent Clearance of Ig-VSG Complexes from the Surface of *Trypanosoma brucei*,
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- Technical Advisor & Alternate Delegate Committee for Guidance & Coordination EEC Science and Technology for Development Research Program (STD-2)
- Member Trinity College Medical Curriculum Review Committee
- Preparation of Revised Curriculum Proposal for TCD Molecular & Cellular Biology Teaching
- Member EEC Committee on University Staff & Student Exchange (European Program)

PUBLICATIONS:

- 1.) Jaffe, J. I., Voorheis, H. P. & McCormack Jr., J. J. (1969) *Trans. Roy. Soc. Trop. Med. Hyg.* 63, 118-119.
'Comparative Effects of Diverse Mamallian Sera on the Uptake and Protein Incorporation of ^{14}C -labelled Amino Acids by Bloodstream Forms of *T. brucei* and *T. rhodesiense* *in vitro*'
- 2.) Voorheis, H. P. (1969) *Trans. Roy. Soc. Trop. Med. Hyg.* 63, 122-123.
'The Effect of *T. brucei* (S-42) on Host Carbohydrate Metabolism: Liver Production and Peripheral Tissue Utilization'
- 3.) Hubbard, R. W., Theriault, D. G. & Voorheis, H. P. (1969) *Bull. N. J. Acad. Sci. Special Symp. Issue*, 52-58.
'The Turnover of Lipid Esters in Adipose Tissue from Cold-Exposed Rats'
- 4.) Hubbard, R. W., Voorheis, H. P. & Theriault, D. G. (1970) *Lipids* 5, 114-120.
'The Incorporation of Glycerol into the Glyceride-Glycerol of Fat Cells Isolated from Chronically Cold-Exposed Rats'
- 5.) Voorheis, H. P. (1971) *Trans. Roy. Soc. Trop. Med. Hyg.* 65, 241-243.
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- 6.) Voorheis, H. P. (1972) *Abstr. Commun. Meet. Fed. Eur. Biochem. Soc.* 8, 96.
'An Amino Group at the Binding Site of the N_1 Amino Acid Transport Carrier of *Trypanosoma brucei*'
- 7.) Voorheis, H. P. (1973) *Trans. Roy. Soc. Trop. Med. Hyg.* 67, 256-257.
'Studies on the Active Site of the N_1 Amino Acid Transport Carrier of *T. brucei*'
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- 12.) Voorheis, H. P., Gale, J. S., Owen, M.J. & Edwards, W. (1979) *Biochem. J.* 180, 11-24.
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'Changes in the Pattern of Cell Surface Proteins During Transformation of Bloodstream Forms of *Trypanosoma brucei* in vitro'
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'Divalent Metal Ion-Dependent Depolymerization of the Pellicular Microtubules from *Trypanosoma brucei*: Experimental Behavior and Kinetic Properties'

Exhibit B

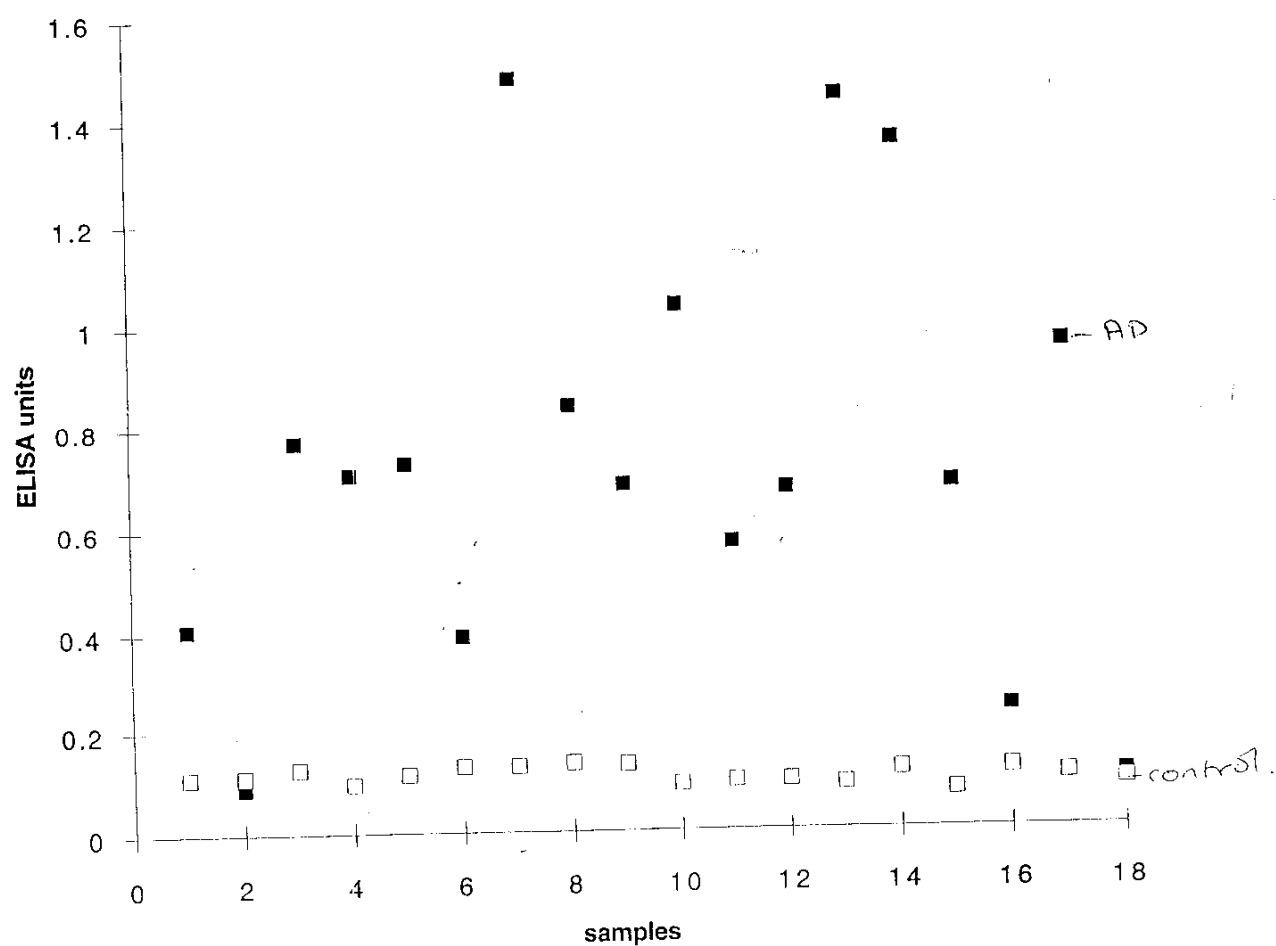
Protocol for ELISA for *tau* fragments

1. Coat Dynatech Immulon microtitre plates with a 1:2000 dilution of the *tau* - 1 antibody (Boehringer) in 0.1M Tris/HCl buffer, pH 7.4 (100 μ l per well). Incubate overnight at 4°C. (Some wells are coated with buffer alone, to measure nonspecific binding). All incubations take place in a humidified chamber. *recognizes N-terminal.*
2. Wash the plates 4 times, using a microwell washer, with 0.1M Tris/HCl buffer, pH 7.4, containing 0.05% (w/v) Tween 20.
3. Block the plates by adding 1 mg/ml bovine serum albumin (200 μ l per well) in 0.1M Tris/HCl, pH 7.4, containing 0.05% Tween 20. This solution is used as the diluent in all subsequent incubations. Incubate for 40 min at room temperature (21°C)
4. Wash as in 2 above.
5. Make a series of dilutions of the serum samples as required (3 dilutions, from 1:25, 1:50, 1:100 and 1:200 were made in this case). 100 μ l of each is pipetted into each well, in triplicate. Standards (e.g. human brain supernatant) and BSA are also added to wells, in triplicate, to assess backgrounds and to check if the assay is working. Incubate 90 min at room temperature.
6. Wash as in 2 above.
7. Incubate wells with a 1:2000 dilution of a rabbit anti-*tau* polyclonal antibody (Sigma, product #T 6402; 100 μ l per well). Incubate 90 min at room temperature.
8. Wash as in 2 above.
9. Incubate wells with a 1:10000 dilution of a goat anti-rabbit alkaline phosphatase conjugate (Sigma, product # A3937, 100 μ l per well). Incubate 90 min at room temperature as before.
10. Wash as in 2 above.
11. Development:
100 μ l of the substrate solution is added to each well. This solution consists of 10% (v/v) diethanolamine buffer, pH 9.8, containing 0.5M $MgCl_2$ and 6mM *p*-nitrophenol phosphate (Sigma Phosphatase Substrate, product #104-0). Allow 75 min for development at room temperature. The reaction is stopped by the addition of 100 μ l 1.5M NaOH to each well, and the absorbance is read at 405 nm in a microwell plate reader.

Exhibit C

Tau expt1 Chart 1

Detection of Tau fragments in AD and control serum



Tau expt1

	AD	Control	
MILD	0.408	0.11	
	0.09	0.111	
	0.772	0.127	
	0.708	0.096	
	0.73	0.114	
	0.391	0.129	
MODERATE	1.482	0.129	
	0.842	0.134	
	0.686	0.13	
	1.038	0.091	
	0.571	0.096	
	0.677	0.096	
SEVERE	1.446	0.089	
	1.357	0.116	
	0.685	0.075	
	0.241	0.117	
	0.96	0.105	
	0.104	0.092	



**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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08/159,969 11/30/93 VOORHEIS

H 4697059

DUFFY, P EXAMINER

18N1/0120

PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036-2711

ART UNIT PAPER NUMBER

1802

12

DATE MAILED: 01/20/95

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☐ This application has been examined ☒ Responsive to communication filed on 11 Oct 1994 ☒ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- ☐ Notice of References Cited by Examiner, PTO-892.
- ☐ Notice of Draftsman's Patent Drawing Review, PTO-948.
- ☒ Notice of Art Cited by Applicant, PTO-1449.
- ☐ Notice of Informal Patent Application, PTO-152.
- ☐ Information on How to Effect Drawing Changes, PTO-1474.
- ☐

Part II SUMMARY OF ACTION

- ☒ Claims 1-3 and 5-7 are pending in the application.
Of the above, claims _____ are withdrawn from consideration.
- ☒ Claims 4 have been cancelled.
- ☐ Claims _____ are allowed.
- ☒ Claims 1-3 and 5-7 are rejected.
- ☐ Claims _____ are objected to.
- ☐ Claims _____ are subject to restriction or election requirement.
- ☒ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
- ☐ Formal drawings are required in response to this Office action.
- ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
- ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
- ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
- ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
- ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
- ☐ Other

EXAMINER'S ACTION

Serial Number: 08/159969

-2-

Art Unit: 1802

1. The amendment filed October 28, 1994 has been entered into the record. Claim 4 has been cancelled. New claims 6 and 7 have been added. Claims 1-3 and 5-7 are pending.
2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
3. Rejection of claims 1-5 under 35 U.S.C. § 101 is withdrawn.
4. Rejection of claims 2, 3 and 5 under 35 U.S.C. § 103 is withdrawn.
5. The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to adequately describe and/or provide an enabling disclosure for the invention being claimed. See Paper No. 7 pages 3-4.
6. Claims 1-3 and 5 are rejected under 35 U.S.C. § 112, first paragraph are maintained for reasons of record as set forth in the objection to the specification in paper No. 7.
7. New claims 6 and 7 are rejected under 35 U.S.C. § 112, first paragraph as set forth in the objection to the specification in paper No. 7.

Serial Number: 08/159969

-3-

Art Unit: 1802

Applicants arguments and declarations have been fully considered but are not persuasive to overcome the rejection of claims 1-3 and 5 made under 35 U.S.C. § 112. In response to this rejection Applicant has filed under Rule 132 two declarations one from the inventor Dr. Paul Voorheis and another from Dr. Davis Coakley. The declaration of Dr. Coakley fails to be persuasive because it is unclear if the samples of blood obtained for analysis had a **definitive** diagnosis of Alzheimer's disease. The definitive diagnosis of Alzheimer's disease as set forth in p 940 Table I section VI. of Exhibit B, McKhann et al., requires both the presence of clinical criteria for **probable** Alzheimer's disease and histopathological evidence obtained from a biopsy or autopsy. In the absence of a correlation of a definitive diagnosis of Alzheimer's disease using recognized criteria with the presence of *tau*-peptides, the methods disclosed appear to be directed to screening methods which aid in diagnosing Alzheimer's disease. Even if the samples provided by Dr. Coakley had met these criteria, the immunoassay of Dr. Voorheis (Exhibit B) fails to distinguish between the *tau*-peptide(s) and the intact *tau*-protein. Because an immunoassay for *tau*-peptides is being claimed, (see Claim 1) the immunoassay must distinguish between the peptides and the intact protein and support for this distinction must be found in the specification as originally filed. As set forth in paper No. 7., the specification does not teach how to assay a sample of blood or cerebrospinal fluid for the presence of anti-*tau*-peptide autoantibody and does not positively correlate the

Serial Number: 08/159969

-4-

Art Unit: 1802

presence of the autoantibody with a **definitive** diagnosis of Alzheimer's disease. There is no teachings of prior art or in the record that anti-tau-peptide autoantibodies even exist in Alzheimer's patients in sufficient quantities in the cerebrospinal fluid or blood can be assayed. There is no evidence of record in the specification as originally filed, that a change in the amount of detected tau-peptide or anti-tau-peptide autoantibody in a sample of blood or cerebrospinal fluid that can be correlated with a change in the cognitive abilities of the patient. The specification fails to teach a) the concentration of tau-peptide or anti-tau-peptide autoantibody that is indicative of Alzheimer's disease and b) how this concentration related to "the status of the patient's Alzheimer's Disease". Thus without further guidance to these aspects, it would take an **undue amount of experimentation** to practice the invention as being claimed.

8. Claims 1 and 3 rejected under 35 U.S.C. § 102(b) as anticipated by Wolozin et al. (Ann. Neurol., 22:521-526, 1987) in light of Ksiezak-Reding et al., (J. Neurosci. Res., 25:412-419, 1990) or alternatively under 35 U.S.C. § 103 are maintained for reasons of record. Applicants arguments have been fully considered but are not persuasive to overcome rejections established in paper No. 7.

Applicant urges that the ALZ-50 monoclonal antibody of Ksiezak-Reding binds only to the intact *tau*-protein because no binding to a N-terminal peptide fragment less than 38kD was observed on an immunoblot. To respond, a careful reading of

Serial Number: 08/159969

-5-

Art Unit: 1802

Ksiezak-Reding reveals that based on other " information and our present results, we conclude that ALZ-50 epitope is located within the first 108 amino acids of bovine tau, or within the first 60 amino acids of fetal human tau" (p 418, col 1, fourth complete paragraph). Ksiezak-Reding proposed that the failure to observe fragments less than 38kD on the immunoblot was due to protease digestion of the N-terminal epitope recognized by ALZ-50 (p 414, Figure 1).

Applicant submits that the A68 protein of Wolozin present in brain tissue and cerebrospinal fluid has not been shown to be elevated in Alzheimer's patients. To respond, Wolozin reveals that the total amount of immunocytochemical staining correlated with the total amount of immunoreactivity seen by ELISA and ALZ-50 western blots confirm that the elevated immunoreactivity in Alzheimer's disease tissue corresponds to the 62-68kD doublet (p 523, col 2 - p 524, col 1 and p 521, col 1, lines 1-7). Wolozin particularly points out that " the selective elevation of the abundance of A68 in Alzheimer tissue suggests its usefulness for diagnostic testing" (p 525, first full paragraph). Wolozin also points out that the volume of CSF required by the western blot could be reduced if a sandwich type immunoassay was employed using a second anti-A68 antibody in combination with ALZ-50 (p 526, col 1, first paragraph). Thus, Wolozin establishes a correlation between increased ALZ-50 immunoreactivity and presence of a 68kD protein in Alzheimer's patients using two different immunoassay methods.

Serial Number: 08/159969

-6-

Art Unit: 1802

New Grounds of Rejection

9. Claims 6 and 7 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification in paper No. 7 and maintained in this Office Action.

10. Claims 1 and 2 are rejected under 35 U.S.C. § 112, first paragraph, for failing to adequately describe and /or provide an enabling disclosure for the invention as now claimed.

There is no evidence of record that a change in the amount of detected *tau*-peptide or anti-*tau*-peptide autoantibody in a blood or cerebrospinal fluid sample correlates with a change in cognitive abilities of the patient diagnosed as having probable Alzheimer's disease so as to allow the physician to "evaluate the status of the patient's Alzheimer's Disease". The specification as originally filed does not teach how to evaluate the status of a patient's Alzheimer's disease and what concentrations of *tau*-peptide or anti-*tau*-peptide autoantibody would correlate with these different "status" of disease. Thus, without further guidance to these aspects, it would take an undue amount of experimentation to practice the invention as now claimed.

11. Claims 1 and 2 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Serial Number: 08/159969

-7-

Art Unit: 1802

Claims 1 and 2 are vague and indefinite because it is unclear what is meant by "status".

12. Applicant's amendment necessitated the new grounds of rejection. Accordingly, **THIS ACTION IS MADE FINAL.** See M.P.E.P. § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 1.136(a).

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Patricia A. Duffy whose telephone number is (703) 305-7555.

Serial Number: 08/159969

-8-

Art Unit: 1802

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Carol A. Spiegel
CAROL A. SPIEGEL
PRIMARY EXAMINER
GROUP 1800

~~CAROL A. SPIEGEL~~
~~PRIMARY EXAMINER~~
~~GROUP 1800~~

pad
January 18, 1995

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, H. Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: Duffy, P.

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

PETITION FOR EXTENSION OF TIME UNDER 37 CFR § 1.136(a)

Assistant Commissioner for Patents
Washington, D.C. 20231RECEIVED
JUN 21 1995
GROUP 1800

Sir:

It is respectfully requested that the time for response to the Office Action dated January 20, 1995, be extended for a period of 2 month(s) from April 20, 1995 to and including June 20, 1995.

The fee for this extension is estimated to be \$ 360.00. Please charge the required fee to Pennie & Edmonds Deposit Account No. 16-1150.

Respectfully submitted,

Date June 20, 1995

By Samuel B. Abrams 30,605
S. Leslie Misrock 18,872
S. Leslie Misrock (Reg. No.)
PENNIE & EDMONDS
1155 Avenue of the Americas
New York, N.Y. 10036-2711
(212) 790-9090

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being filed with the United States Patent and Trademark Office by facsimile transmission on

June 20, 1995(date) to facsimile telephone number 1-703-378-4065*Samuel B. Abrams*
Samuel B. Abrams

30,605

(Reg. No.)

PENNY2-38372.1

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16-1150 110 116

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, H. Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: Duffy, P.

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

NOTICE OF APPEAL FROM THE PRIMARY EXAMINER
TO THE BOARD OF PATENT APPEALS AND INTERFERENCESAssistant Commissioner for Patents
Box AF
Washington, D.C. 20231

Sir:

Applicant hereby appeals to the Board of Patent Appeals and Interferences from the decision dated January 20, 1995, of the Primary Examiner finally rejecting claims 1 and 3 of the above-identified application.

The item(s) checked below are appropriate:

1. ☒ An extension of time for responding to the final rejection for 2 month(s):
☐ was filed on
☒ is submitted herewith.
2. ☒ A timely response to the final rejection has been filed.
3. ☒ A fee in the amount of \$280.00 is:
☒ Required.
☐ Not required (Fee paid in prior appeal).
4. ☒ Please charge the required fee, if any, to Pennie & Edmonds Deposit Account No. 16-1150.

RECEIVED

JUN 21 1995

GROUP 1800

Date June 20, 1995

Respectfully submitted

By Samuel B. Abrams 30,605S. Leslie Misrock 18,872
(Reg. No.)S. Leslie Misrock
PENNIE & EDMONDS
1155 Avenue of the Americas
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June 20, 1995

(date) to facsimile telephone number 1-703-308-4065

Samuel B. Abrams
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30,605

(Reg. No.)

PENY2343313.1

MH11084 06/29/95 08159969

16-1150 110 119 280.00CH

JUN 20 '95 16:02 FR PENNIE EDMOND

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, H. Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: Duffy, P.

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

AMENDMENT UNDER 37 CFR § 1.116

Assistant Commissioner for Patents
Box AF
Washington, D.C. 20231

Sir:

Please consider the following amendments and remarks, which are responsive to the Office Action mailed January 20, 1995. Enclosed herewith is a Petition to Extend Time Under 37 CFR § 1.136(a) and a Notice of Appeal.

AMENDMENTS

In the Claims:

Please cancel claims 2, 5-7 without prejudice.

Please amend the claims as follows:

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being filed with the United States Patent and Trademark Office by facsimile transmission on

June 20, 1995

(date) to facsimile telephone number 1-703-308-4065


Samuel B. Abrams

30,605

(Reg. No.)

PENY2-366518.1

1. (Twice Amended) A method for confirming a clinical diagnosis of [diagnosing] Alzheimer's Disease in a patient suspected of having Alzheimer's Disease, comprising assaying a sample of blood [or cerebrospinal fluid] obtained from the patient for a concentration of a tau-peptide using either an anti-tau-peptide antibody or an anti-tau-peptide Fab fragment, wherein further the tau-peptide is derived from the amino terminal 200 amino acids or carboxy terminal 50 amino acids of a tau-protein; and employing the assayed concentration to evaluate [the status of] the patient's clinical diagnosis of Alzheimer's Disease.

2
3. (Twice Amended) The method of claim[s] 1 [or 2], wherein the assaying step comprises performing an assay selected from the group consisting of a radioimmunoassay, an enzyme-linked immunosorbant assay, a sandwich assay, a precipitin reaction, a gel immunodiffusion assay, an agglutination assay, a fluorescent immunoassay, a protein A immunoassay and an immunoelectrophoresis assay.

REMARKS

Claims 1 and 3 are in the case.

Claims 1 and 3 have been amended to expedite the prosecution. The preamble of claim 1 has been amended to replace the phrase "diagnosing Alzheimer's Disease" by the phrase "confirming the clinical diagnosis of Alzheimer's Disease." Claim 1 has been further amended by replacing the phrase "evaluate the status of the patients's Alzheimer's Disease" with the phrase "evaluate the patient's clinical

diagnosis of Alzheimer's Disease." Written description support for these amendments can be found at page 13, lines 6-7.

Claim 1 has been amended to delete cerebrospinal fluid from the scope of the claim.

Claim 3 has been amended to remove reference to canceled claim 2.

Applicant respectfully submits that the foregoing amendments obviate the following rejections of the Office Action.

The Office Action rejected claim 1-3 and 5 under 35 U.S.C. § 112, first paragraph. The Office Action alleged that the Applicant has not exemplified a method of diagnosing Alzheimer's Disease, which was recited by the preambles to claims 1 and 2, because a definitive diagnosis of Alzheimer's Disease requires histopathologic examination and because the Applicant had not presented data showing the correlation of the results of an assay performed according to the invention with the results of a histopathologic examination.

Applicant respectfully submits that the foregoing rejection is insufficient as a matter of law. The alleged absence of specific exemplification of a claimed embodiment does not create a presumption of inoperability or of non-enablement. See, e.g., *In re Angstadt and Griffin*, 190 USPQ 214, 218 (CCPA 1976) (35 U.S.C. § 112, first paragraph does not require applicants to test every species within scope of claims in an unpredictable area).

The Examiner has provided no other evidence which is even asserted to show that one of ordinary skill would

reasonably doubt the disclosed utility that is expressed in the preamble. Therefore, the Examiner has not met the Examiner's initial burden, under 35 U.S.C. § 112, and the rejection is improper. *In re Brana*, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (following *In re Marzocchi* 169 USPQ 367, 369 (CCPA 1971) (an application that teaches the manner and process of making and using an invention, in terms that correspond to the scope of the claims, is entitled to be presumed to meet the requirements of 35 U.S.C. § 112, first paragraph, until the examiner presents contrary evidence)).

However, to expedite prosecution, the preamble of claim 1 has been amended to respond to the Examiner's concerns. Claim 1, as amended, recites that the method is directed to confirmation of a clinical diagnosis of Alzheimer's Disease. Applicant respectfully submits that the Declarations Under Rule 132, which were submitted with the Applicant's Amendment, mailed October 17, 1994, exemplify the use of the invention to confirm a clinical diagnosis of Alzheimer's Disease.

The Declaration of Dr. Davis Coakley, M.D., Under 37 CFR § 1.132 (the "Coakley Declaration") at ¶ 5 states that samples of blood from 18 "patient" subjects and 18 "control" subjects were made available to Dr. Voorheis for assay. The Coakley Declaration at ¶ 6 states that diagnoses of Alzheimer's Disease were made according to the clinical and neuropsychological assessments as set forth in Table 1, p. 940 of McGhann et al., which was attached as Exhibit B to the Coakley Declaration. Each of the 18 patient subjects and none

of the control subjects had been diagnosed as having Alzheimer's Disease by these criteria.

The Declaration of H. Paul Voorheis, M.D., Ph.D., Under Rule 132 (the "Voorheis Declaration") reports the results of the assay for r antigen of the samples of blood that had been provided by Dr. Coakley and his colleagues. The results are presented as a table in Exhibit B of the Voorheis Declaration, as a histogram in Exhibit C of the Voorheis Declaration and are discussed in ¶ 6 of the Voorheis Declaration. The results show that the assay of the invention divided the samples into two distinct populations which consist of: i) all the control samples and two patient samples, Nos. 2 and 18, and ii) all the remaining patient samples, i.e., Nos. 1 and 3-17.

As stated in ¶ 8 of the Voorheis Declaration, the observed statistical correlation between the clinical diagnosis of Alzheimer's Disease, provided by Dr. Coakley, and the blood levels of r antigen was as close as could be expected in view of the not infrequent discordance between the clinical and the histopathological (definitive) diagnoses.

In the exemplification presented, the assays performed according to the invention have confirmed the clinical diagnoses of patients Nos. 1 and 3-17 and have not confirmed the clinical diagnosis of Alzheimer's Disease of patients Nos. 2 and 18 because the assays show that the blood from these patients has a r antigen level that is characteristic of subjects not having Alzheimer's Disease. Applicant respectfully submits that the Voorheis and Coakley Declarations demonstrate that assays performed according to

the teaching of the Application are operable to confirm the clinical diagnosis of Alzheimer's Disease.

The Office Action did not allege the invention is inoperable or lacks patentable utility; at page 3, it stated that the method of the invention concerns "screening methods which aid in diagnosing Alzheimer's Disease." Rather, the Office Action alleged that the utility of the invention was misdescribed by the preamble of the claims. Applicant respectfully submits that the preamble, as amended, describes the utility that was identified in the Office Action in language corresponding to that of the specification. The amendment to claim 1 therefore obviates the rejection under 35 U.S.C. § 112, first paragraph, for an alleged disparity between the results obtained by the method that is claimed and that which is described in the preamble.

The Examiner has rejected claim 1 under 35 U.S.C. § 112, first paragraph, because the step of assaying the concentration of τ peptide was alleged not to be enabled. More specifically, the Examiner alleged that assays performed according to the Applicant's disclosure detect both τ protein and τ peptides, and that, therefore, assaying a τ peptide, independently of τ protein, was not enabled.

Applicant submits that the rejection is moot because claim 1, as amended, is directed to the assay of samples of blood. It is believed that the concentration of τ protein in the blood of both Alzheimer's patients and normals is negligible. Therefore, the alleged inability of the assays taught by the specification to distinguish τ protein from τ peptides does not affect the operability of the invention to

measure the τ peptide concentration in the blood of a patient suspected of having Alzheimer's Disease.

The Examiner has rejected claim 2 under 35 U.S.C. § 112, first paragraph, in view of an alleged lack of exemplification of the use of anti-tau-peptide autoantibodies for the method of the invention. The rejection is obviated by the cancellation of claim 2.

Claim 1 was rejected under 35 U.S.C. § 112, first paragraph because of the phrase "status of the patient's Alzheimer's Disease." The invention was allegedly inoperable to distinguish among patients having Alzheimer's Disease of differing severity. The phrase has been deleted from the claim 1.

The Examiner has further rejected claims 1, 3 and 5 under 35 U.S.C. § 112, first paragraph, because the specification fails to teach a level of τ peptide that is indicative of Alzheimer's Disease.

The rejection is obviated as regards to claim 5 by its cancellation. Applicant traverses the rejection with respect to claims 1 and 3. The data reported by the Voorheis and Coakley Declarations show that an assay performed according to the teaching of the specification clearly divides blood samples into groups having "normal" and "elevated" levels of τ antigen and that elevated levels of τ antigen are positively correlated with the clinical diagnosis of Alzheimer's Disease. Applicant submits that the determination of the particular value that is used to discriminate between the normal and elevated populations can be readily determined without undue experimentation.

Claims 1 and 3 were rejected under 35 U.S.C. §§ 102(b) and 103 over Wolozin et al., (Ann. Neurol. 22:521-26, 1987) in view of Ksiezak-Redding et al., (J. Neurosci. Res. 25:412-19, 1990). Wolozin et al. is alleged to have diagnosed Alzheimer's Disease by detecting an abnormally elevated level of a tau-protein in the CSF of a subject. Claim 1 has been amended to incorporate the limitation of canceled claim 6, which was not rejected over Wolozin et al. in view of Ksiezak-Redding et al. As amended, claim 1 recites that the sample to be assayed is a sample of blood. Neither Wolozin et al. nor Ksiezak-Redding et al. teaches or suggests a method which includes the assay of a sample of blood. Therefore, Applicant submits that the rejection over Wolozin et al. in view of Ksiezak-Redding et al. is obviated by the amendment.

Applicant submits that each of the rejections of the Office Action is obviated or overcome by the foregoing amendments to claims in view of these arguments. Applicant urges that claims 1 and 3, as amended, are in condition for allowance and earnestly requests that the Examiner reconsider and withdraw all rejections and pass the claims to allowance.

Respectfully submitted,

Date JUNE 20, 1995

By Samuel B. Adams 30,605
S. Leslie Misrock 18,872
S. Leslie Misrock (Reg. No.)

PENNIE & EDMONDS
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosure

06/20/95 TUE 16:01 FAX 703 308 4065

GP 1800 FAX 5

001

*** RX REPORT ***

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TX/RX NO	5303	
CONNECTION TEL		212 869 9741
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NEW YORK, NEW YORK 10036Telephone: (212) 790-9090
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JUN 21 1995

GROUP 1800

TO: Assistant Commissioner for Patents
Box AF
Washington, DC 20231

ATTN: P. Duffy

FAX NO.: 1-703-308-4065

DATE: June 20, 1995

FROM: Samuel B. Abrams

PAGES: 11 (including cover page) OUR REF.: 4697-059

We are transmitting herewith a PETITION FOR
EXTENSION OF TIME UNDER 37 CFR § 1.136(a); a NOTICE OF APPEAL
FROM THE PRIMARY EXAMINER TO THE BOARD OF PATENT APPEALS AND
INTERFERENCES and an AMENDMENT UNDER 37 CFR § 1.116 for filing
in:

Application of: Voorheis, H. Paul

Serial No.: 08/159,969

Filed: November 30, 1993

Group Art Unit: 1802

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

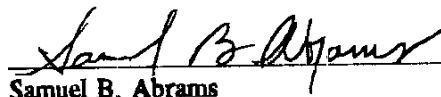
Examiner: P. Duffy

Attorney Docket No.: 4697-059

This transmission was sent from our GIII RAPICOM
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CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office
on the date shown below.


Samuel B. Abrams30.605
(Reg. No.)

Date: June 20, 1995

PENY2-216673.1



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
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08/159,969 11/30/93 VOORHEIS

H 4697059

EXAMINER
DUFFY, F

18N1/0721

PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036-2711

ART UNIT PAPER NUMBER

DATE MAILED 1802

EXAMINER INTERVIEW SUMMARY RECORD

07/21/95

All participants (applicant, applicant's representative, PTO personnel):

- (1) PATRICIA A. DUFFY (Exr) (3) _____
(2) Samuel Abrams (Applicants Rep) (4) _____

Date of interview 7/12/95

Type: ☒ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No. If yes, brief description: _____

Agreement ☒ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: 1 + 3

Identification of prior art discussed: NONE

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Discussed claim language changes which would define the method method steps of the invention more clearly. Mr. Abrams agreed to indicate the changes were agreeable.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1-7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☒ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Patricia A. Duffy
Examiner's Signature

PTOL-413 (REV. 1-84)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
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08/159,969 11/30/93 VOORHEIS

H 4697059

EXAMINER
DUFFY, J.

18N1/0721

PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036-2711

ART UNIT PAPER NUMBER

1802

DATE MAILED:

07/21/95

NOTICE OF ALLOWABILITY

PART I.

- ☒ This communication is responsive to June 01, 1995 After final Amendment
- ☒ All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course.
- ☒ The allowed claims are 1 and 3
- ☐ The drawings filed on _____ are acceptable.
- ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received. ☐ not been received. ☐ been filed in parent application Serial No. _____, filed on _____
- ☒ Note the attached Examiner's Amendment.
- ☒ Note the attached Examiner Interview Summary Record, PTOL-413.
- ☐ Note the attached Examiner's Statement of Reasons for Allowance.
- ☐ Note the attached NOTICE OF REFERENCES CITED, PTO-892.
- ☐ Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

- ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.
- ☒ APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.
 - ☒ Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. 4. CORRECTION IS REQUIRED.
 - ☐ The proposed drawing correction filed on _____ has been approved by the examiner. CORRECTION IS REQUIRED.
 - ☐ Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.
 - ☒ Formal drawings are now REQUIRED.

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

Attachments:

- ☒ Examiner's Amendment
- ☒ Examiner Interview Summary Record, PTOL-413
- ☐ Reasons for Allowance
- ☐ Notice of References Cited, PTO-892
- ☐ Information Disclosure Citation, PTO-1449

- ☐ Notice of Informal Application, PTO-152
- ☐ Notice re Patent Drawings, PTO-948
- ☐ Listing of Bonded Draftsmen
- ☐ Other

Serial Number: 08/159,969

-2-

Art Unit: 1802

Part III EXAMINER'S AMENDMENT

1. An Examiner's Amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 C.F.R. § 1.312. To ensure consideration of such an amendment, it **MUST** be submitted no later than the payment of the Issue Fee.

Authorization for this Examiner's Amendment was given in a telephone interview with Samuel Abrams on July 13, 1995.

2. The application has been amended as follows:

In the title:

Diagnostic method for Alzheimer's Disease by screening for tau-peptides in the blood of a patient.

In the claims:

1. (amended) A method for confirming a clinical diagnosis of Alzheimer's Disease in a patient suspected of having Alzheimer's Disease, comprising [assaying a sample of blood obtained from the patient for a concentration of tau-peptide using either an anti-tau-peptide antibody or an anti-tau-peptide Fab fragment] performing an assay to determine the concentration of tau-peptide in the patient's blood, wherein the assay comprises contacting a sample of blood obtained from the patient with an antibody which specifically binds tau-peptide or a Fab fragment which specifically binds tau-peptide, wherein further the tau-peptide is derived from the amino terminal 200 amino acids or

Σ

44

Serial Number: 08/159,969

-3-

Art Unit: 1802

Σ¹
carboxy terminal 50 amino acids of a tau-protein; and [employing the
assayed concentration to evaluate the patient's] determining the
concentration of tau-peptide wherein an elevated concentration of tau-
peptide confirms the clinical diagnosis of Alzheimer's Disease.

Σ²
2. (amended) The method of claim 1, wherein the [assaying step comprises performing]

assay comprises an assay selected from the group consisting of a
radioimmunoassay, an enzyme-linked immunosorbant assay, a sandwich
assay, a precipitin reaction, a gel immunodiffusion assay, an agglutination
assay, a fluorescent immunoassay, a protein A immunoassay and an
immunoelectrophoresis assay.

In the abstract:

Deleted ", subtyping and monitoring" in line 2.

3. Any inquiry concerning this communication or earlier communications from the
examiner should be directed to Patricia A. Duffy, Ph.D. whose telephone number is (703)
305-7555.

Any inquiry of a general nature or relating to the status of this general application
should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers relating to this application should be directed may be submitted to Group
1800 by facsimile transmission. The faxing of such papers must conform with the notice

45

Serial Number: 08/159,969

-4-

Art Unit: 1802

published in the Official Gazette, 1096 OG 30 (November 15, 1989). The FAX number for Art Unit 1802 is (703) 308-4065.

Toni R. Scheiner

TONI R. SCHEINER
SUPERVISORY PATENT EXAMINER
GROUP 1800

^{PAD}
Patricia A. Duffy, Ph.D.
July 19, 1995



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: Box ISSUE FEE
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Washington, D.C. 20231

1881/0721

PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036-2711

NOTICE OF ALLOWANCE
AND ISSUE FEE DUE

☐ Note attached communication from the Examiner

☐ This notice is issued in view of applicant's communication filed

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
08/159,969	11/30/93	002	DUFFY, P	1302 07/21/95
First Named Applicant	VOORHEIS, H. PAUL			

TITLE OF INVENTION

DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE BY SCREENING FOR
TALE-PEPTIDES IN THE BLOOD OF A PATIENT (AS AMENDED)

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1 4697059	435-002,920	NS4	UTILITY	NO	\$1210.00	10/30/95

**THE APPLICATION IDENTIFIES ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT.
PROSECUTION ON THE MERITS IS CLOSED.**

**THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS
APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.**

HOW TO RESPOND TO THIS NOTICE:

I. Review the SMALL ENTITY Status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

- If the status is changed, pay twice the amount of the FEE DUE shown above and notify the patent and Trademark Office of the change in status, or
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- Pay FEE DUE shown above, or
- File verified statement of Small Entity Status before, or with, pay of 1/2 the FEE DUE shown above.

II. Part B of this notice should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by charge to deposit account, Part B should be completed and returned. If you are charging the ISSUE FEE to your deposit account, Part C of this notice should also be completed and returned.

III. All communications regarding this application must give series code (or filing date), serial number and batch number. Please direct all communication prior to issuance to Box ISSUE FEE unless advised to contrary.

IMPORTANT REMINDER: Patents Issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B—ISSUE FEE TRANSMITTAL

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE. Blocks 2 through 6 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advance orders and notification of maintenance fees will be mailed to addressee entered in Block 1 unless you direct otherwise, by: (a) specifying a new correspondence address in Block 3 below; or (b) providing the PTO with a separate "FEE ADDRESS" for maintenance fee notifications with the payment of Issue Fee or thereafter. See reverse for Certificate of Mailing.

1. CORRESPONDENCE ADDRESS	2. INVENTOR(S) ADDRESS CHANGE (Complete only if there is a change)
PENNIE & EDMONDS 1155 AVENUE OF THE AMERICAS NEW YORK, NY 10036-2711 18N1/0721	INVENTOR'S NAME
	Street Address
	City, State and ZIP Code
	CO-INVENTOR'S NAME
	Street Address
	City, State and ZIP Code
	☐ Check if additional changes are on reverse side

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
08/159,969	11/30/93	002	DUFFY, P	1802 07/21/95
First Named Applicant	VOORHEIS, H. PAUL			

TITLE OF INVENTION
 DIAGNOSTIC METHOD FOR ALZHEIMER'S DISEASE BY SCREENING FOR TALE-PEPTIDES IN THE BLOOD OF A PATIENT (AS AMENDED)

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL-ENTITY	FEE DUE	DATE DUE
4697059	435-007.920	N54	UTILITY	NO	\$1210.00	10/23/95

3. Correspondence address change (Complete only if there is a change)	4. For printing on the patent front page, list the names of not more than 3 registered patent attorneys or agents OR, alternatively, the name of a firm having as a member a registered attorney or agent. If no name is listed, no name will be printed.
	1 Pennie & Edmonds 2 3

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 AG00359 11/07/95 08159969 16-1150 160 142 1,250.00CH
 AG00360 11/07/95 08159969 16-1150 160 561 300.00CH

5. ASSIGNMENT DATA TO BE PRINTED ON THE PATENT (print or type)		6a. The following fees are enclosed: ☐ Issue Fee ☐ Advance Order - # of Copies _____	
(1) NAME OF ASSIGNEE: Provost, Fellows and Scholars of Trinity College		(b) The following fees should be charged to: DEPOSIT ACCOUNT NUMBER 16-1150 (ENCLOSE PART C) ☒ Issue Fee ☒ Advance Order - # of Copies 100 ☒ Any Deficiencies in Enclosed Fees	
(2) ADDRESS: (CITY & STATE OR COUNTRY) Dublin, Republic of Ireland		The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee to the application identified above.	
A. ☐ This application is NOT assigned. ☐ Assignment previously submitted to the Patent and Trademark Office. ☒ Assignment is being submitted under separate cover. Assignments should be directed to Box ASSIGNMENTS. PLEASE NOTE: Unless an assignee is identified in Block 5, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.		Authorized Signature: S. Leslie Misrock 18,872 (Date) 19 Oct. 1995 NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.	

1. TRANSMIT THIS FORM WITH FEE-CERTIFICATE OF MAILING ON REVERSE

Certificate of Mailing

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to:

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on October 19, 1995
(Date)

Ismael Maldonado
(Name of person making deposit)

[Signature]
(Signature)

(Date) Express Mail No. EM 325 962 348 US

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UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/159,969	11/30/93	VDORHEIS	H 4697059

OF RECORD:

PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036-2711

07N1/0807

DUFFY, R. EXAMINER	
ART UNIT	PAPER NUMBER
1802	18

DATE MAILED:

08/07/95

A statement under Section 152 of Public Law 703 (83rd Congress, 2nd Session), concerning the making of the invention or discovery described therein, has been filed in the above-identified application. A copy of the application and a copy of the statement are forwarded herewith.

A statement of the date of receipt in the U.S. Department of Energy of the copy of the statement and the copy of the application is requested to determine the starting of the 90-day period recited in paragraph 2 of Section 152. An endorsed copy of this letter is provided for this purpose.

By direction of the Commissioner

Special Laws Administration Group

Encl: Photo statement
Photo application
Receipt copy of this letter

Receipt in the U.S. Department of Energy is acknowledged of a copy of the above letter and its enclosure.

Date

THIS CORRESPONDENCE TO:

Assistant General Counsel
for Patents, GC-42
U.S. Department of Energy
1000 Independence Ave.
MS 6F-067
Washington, D.C. 20585

Signed

11/20/95

15:12

009



**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/159969			10097059

OF RECORD:

DATE EXAMINED	
ART UNIT	PAPER NUMBER
1817	19

DATE MAILED:

08/07/95

NOV 20 1995

RECEIVED

A statement under Section 152 of Public Law 703 (83rd Congress, 2nd Session), concerning the making of the invention or discovery described therein, has been filed in the above identified application. A copy of the application and a copy of the statement are forwarded herewith.

A statement of the date of receipt in the U.S. Department of Energy of the copy of the statement and the copy of the application is requested to determine the starting of the 90-day period recited in paragraph 2 of Section 152. An endorsed copy of this letter is provided for this purpose.

By direction of the Commissioner

Special Laws Administration Group

Encl: Photo statement
Photo application
Receipt copy of this letter

THIS APPLICATION HAS BEEN REVIEWED AND
FROM THE INFORMATION AT HAND YOU ARE
HEREBY ADVISED THAT THE U.S. DEPARTMENT
OF ENERGY WILL NOT FILE A DIRECTIVE IN
THIS CASE.

NOVEMBER 20, 1995
DATE

for
INITIALS

Receipt in the U.S. Department of Energy is acknowledged of a copy of the above letter and its enclosure.

NOVEMBER 20, 1995

Date
THIS CORRESPONDENCE TO:

Assistant General Counsel
for Patents, GC-42
U.S. Department of Energy
1000 Independence Ave.
MS 6F-067
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Anne L. Remsburg
Signed for

OFFICE OF THE ASSISTANT GENERAL COUNSEL
FOR TECHNOLOGY TRANSFER AND
INTELLECTUAL PROPERTY



Notice of Allowance and Issue Fee Due: mailed, July 21, 1995
Date of Notice of Allowability: mailed, July 21, 1995
Issue Batch No.: N54
Serial No. 08/159,969

DJM 4100

#28
B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Voorheis, H. Paul

Serial No.: 08/159,969

Group Art Unit: 1802

Filed: November 30, 1993

Examiner: P. Duffy

For: DIAGNOSTIC METHOD FOR
ALZHEIMER'S DISEASE

Attorney Docket No.: 4697-059

SUBMISSION OF FORMAL DRAWINGS

Honorable Official Draftsman
Washington, D.C. 20231

Sir:

Applicants submit herewith two (2) sheets of two (2) drawings in response to the Notice of that "Formal drawings are now REQUIRED" which was included on the Notice of Allowability mailed July 21, 1995 in the above-captioned application.

This submission is timely filed on Monday, October 23, 1995, which is the first business day in the United States Patent and Trademark Office following on or after three months from the mailing date of the Notice. No fee is believed due with this submission. If a fee is required, its payment from Pennie & Edmonds Deposit Account No. 16-1150 is hereby authorized.

Respectfully submitted,
by *[Signature]* 36,156
[Signature] 18,872
S. Leslie Misrock (Reg. No.)

Date October 23, 1995

PENNIE & EDMONDS
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New York, N.Y. 10036-2711
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EXPRESS MAIL CERTIFICATION

"Express Mail" label No. EM325 962 379US Date of Deposit OCTOBER 23, 1995
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

MICHAEL VIGUIE

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(Signature of person making paper or fee)

PENY3-418940.1

696651/80

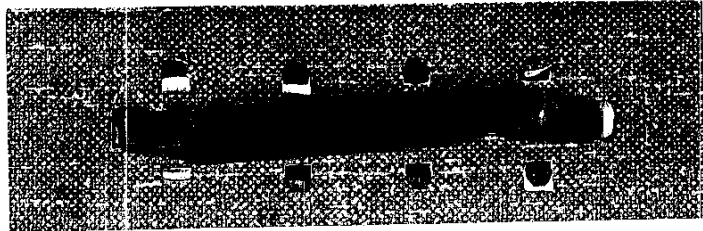
5492812

APPROVED	O.G. FIG
BY	CLASS
DRAFTSMAN	SUBCLASS

-37 CCGCCTCTGCTGACATATCAGGTGAACCTTTCGAACCAGG

1 Met Ala Glu Pro Arg Gln Glu Phe Glu Val Met Glu Asp His Ala Gly Thr Tyr Gly Leu Gly Asp Arg Lys Asp Gln Gly Gly Tyr Thr
1 ATG GCT GAG CCC CCG CAG GAG TTC GAA GTG ATG GAA GAT CAC GCT GGG ACC TAC GGG TTG GGG GAC AGG AAA GAT CAG GGG GGC TAC ACC
31 Met His Gln Asp Gln Glu Gly Asp Thr Asp Ala Gly Leu Lys Ala Glu Glu Ala Gly Ile Gly Asp Thr Pro Ser Leu Glu Asp Glu Ala
91 ATG CAC CAA GAC CAA GAG GCT GAC CAC GCT GAC ACC GAC GCT GGC CTC AAA GCT GAA GAA GCA GGC ATT GGA GAC ACC CCC AGC CTG GAA GAC GAA GCT
61 Ala Gly His Val Thr Gln Ala Arg Met Val Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Lys Lys Ala Lys Gly Ala Asp Gly Lys
101 GCT GGT CAC GTG ACC CAA GCT CCG ATG GTC AGT AAA ACC AAA GAC GGC ACT GGA AGC GAT GAC AAA AAA GCC AAG GGG GCT GAT GGC AAA
91 Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro Pro Ala
271 ACG AAG ATC GCC ACA CCG GCG GGA GCA GCC CCT CCA GCC CAG AAG GGC CAG GCC AAC GGC ACC AGG ATT CCA GCA AAA ACC CCG CCC GCT
121 Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser
361 CCA AAG ACA CCA CCC AGC TCT GGT GAA CCT CCA AAA TCA GCG GAT CCG AGC GAT CCG GGC TAC AGC ACC CCC GGC ACT CCC GGC AGC
151 Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys Lys Val Ala Val Arg Thr Pro Pro Lys Ser Pro Ser
451 CGC TCC CCG ACC CCG TCC CTT CCA ACC CCA CCC ACC CCG GAG CCC AAG AAG GTG GCA GTG CTC CGT ACT CCA CCC AAG TCG CCG TCT TCC
181 Ala Lys Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His
541 GCC AAG ACC CCG CTG CAG ACA GCC CCC GTG CCC ATG CCA GAC CTG AAG AAT GTC AAG TCC AAG ATC GGC TCC ACT GAG AAC CTG AAG CAC
211 Gln Pro Gly Gly Gly Lys Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His
631 CAG CCG GCA GCG GCG AAG GTG CAA ATA GTC TAC AAA CCA GTT GAC CTG AGC AAG GTG ACC TCC AAG TGT GGC TCA TTA GGC AAC ATC CAT

FIG.1A



PTO UTILITY GRANT

Paper Number 24

The
United
States
of
America

The Commissioner of Patents
and Trademarks

*Has received an application for a patent
for a new and useful invention. The title
and description of the invention are en-
closed. The requirements of law have
been complied with, and it has been de-
termined that a patent on the invention
shall be granted under the law.*

Therefore, this

United States Patent

*Grants to the person or persons having
title to this patent the right to exclude
others from making, using or selling the
invention throughout the United States
of America for the term of seventeen
years from the date of this patent, sub-
ject to the payment of maintenance fees
as provided by law.*



Bruce Lehman
Commissioner of Patents and Trademarks
L. P. Holton

Attest

PTO-1584

FPI-LOM

(RIGHT INSIDE)

PATENT APPLICATION FEE DETERMINATION RECORD						Application or Docket Number									
Effective October 1, 1992						159,969									
CLAIMS AS FILED - PART I						SMALL ENTITY		OR		OTHER THAN SMALL ENTITY					
FOR		(Column 1)		(Column 2)		RATE		FEE		RATE		FEE			
BASIC FEE								\$355.00		OR		\$710.00			
TOTAL CLAIMS		5		minus 20 = *		x\$11=				OR		x\$22=			
INDEPENDENT CLAIMS		4		minus 3 = *		x 37=				OR		x 74= 74			
MULTIPLE DEPENDENT CLAIM PRESENT						+115=				OR		+230= 230			
						TOTAL				OR		TOTAL 1,014			
* If the difference in column 1 is less than zero, enter "0" in column 2															
CLAIMS AS AMENDED - PART II						SMALL ENTITY		OR		OTHER THAN SMALL ENTITY					
C		(Column 1)		(Column 2)		(Column 3)		RATE		ADDITIONAL FEE		RATE		ADDITIONAL FEE	
AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT				HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA							
Total		7		Minus		** 20		=		x\$11=		OR		x\$22=	
Independent		4		Minus		*** 4		=		x 37=		OR		x 74=	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM										+ 115=		OR		+230=	
						TOTAL				OR		TOTAL			
						ADDIT. FEE				OR		ADDIT. FEE			
AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT				HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA							
Total		*		Minus		**		=		x\$11=		OR		x\$22=	
Independent		*		Minus		***		=		x 37=		OR		x 74=	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM										+ 115=		OR		+ 230=	
						TOTAL				OR		TOTAL			
						ADDIT. FEE				OR		ADDIT. FEE			
AMENDMENT C		CLAIMS REMAINING AFTER AMENDMENT				HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA							
Total		*		Minus		**		=		x\$11=		OR		x\$22=	
Independent		*		Minus		***		=		x 37=		OR		x 74=	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM										+115=		OR		+230=	
						TOTAL				OR		TOTAL			
						ADDIT. FEE				OR		ADDIT. FEE			
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.															
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".															
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".															
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.															

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET (FOR USE WITH FORM PTO-875)							SERIAL NO.		FILING DATE	
							APPLICANT(S) 159,969			
CLAIMS										
	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT			*	*	*
	IND.	DEP.	IND.	DEP.	IND.	DEP.		IND.	DEP.	IND. DEP.
1	1						51			
2	1						52			
3		2					53			
4	1						54			
5	1						55			
6							56			
7							57			
8							58			
9							59			
10							60			
11							61			
12							62			
13							63			
14							64			
15							65			
16							66			
17							67			
18							68			
19							69			
20							70			
21							71			
22							72			
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24							74			
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36							86			
37							87			
38							88			
39							89			
40							90			
41							91			
42							92			
43							93			
44							94			
45							95			
46							96			
47							97			
48							98			
49							99			
50							100			
TOTAL IND.	4						TOTAL IND.			
TOTAL DEP.	1						TOTAL DEP.			
TOTAL CLAIMS	5						TOTAL CLAIMS			