

**Immunology Devices
Panel
July 15, 2005**

**Immunology Devices Panel Meeting
July 15, 2005
Holiday Inn, Gaithersburg, Maryland**

**Panel Chair: Clive Taylor, M.D., D.Phil.
Executive Secretary: Rufina Carlos**

**Nymox Urine Neural Thread Protein (NTP) Kit (P040010)
Agenda***

- | | | |
|-------------|---|---------------------|
| 8:30 – 8:45 | Call to Order | Chairperson |
| | Opening Remarks..... | Executive Secretary |
| 8:45 -9:15 | Special Topics | |
| | • Sousan Altaie, PhD. – Critical Path Initiative | |
| | • Susan Gardner, PhD. –Role of OSB in the review of post market study designs | |
| 9:15- 9:45 | Open Public Hearing** | |
| 9:45-10:00 | Break | |
| 10:00-11:15 | Sponsor Presentation | |
| | • Ira Goodman, MD – Chairman, Dept of Neurology, Orlando Regional Healthcare System | |
| | • Patrisio Reyes, MD – Director, Alzheimer’s Disease and Cognitive Disorders, Neurological Institute, Phoenix | |
| | • Paul Averback MD, DABP (Neuropath),- President, Nymox | |
| | • Ralph Richter, MD,- University of Oklahoma, College of Medicine | |
| 11:15-12:30 | FDA Presentation | |
| | • Robert L. Becker, MD, PhD,- Director, Division of Immunology and Hematology Devices | |
| | • Marina Kondratovich, PhD, - Statistician, CDRH/OSB/DBS/DDB | |
| | • Ranjit Mani, MD, - Medical Reviewer (Neurology),CDER/OND/DNP | |
| | • Robert L. Becker - Summary Statement | |
| 12:30 -1:15 | Lunch Break | |
| 1:15 – 3:15 | Panel Discussion | |
| 3:15 -3:30 | Break | |

3:30-4:00 **Open Public Hearing****
4:00-5:00 **Panel Deliberations and Vote**

5:00 **Adjourn**

* All times are approximate

** **Open Public Hearings**-Interested persons may present data, information, or views, orally or in writing, on the meeting agenda. Scheduled speakers who have requested time to address the panel will speak at this time. After they have spoken, Dr. Taylor will recognize unscheduled speakers as time allows. Only the panel may question speakers during the open public hearing.

Immunology Devices Panel Roster

July 15, 2005

<u>Name and Specialty</u>	<u>Affiliation</u>	<u>Role</u>
Clive R. Taylor, M.D., D. Phil. Immunopathology	University of Southern California Los Angeles, California	Chair
Susanne M. Gollin, Ph.D. Genetics/Tumor Markers	University of Pittsburgh Pittsburgh, Pennsylvania	Voting Member
James L. Gulley, M.D., Ph.D. Immunotherapy	National Cancer Institute, NIH Bethesda, Maryland	Voting Member
Terrance R. Lichtor, M.D., Ph.D. Neurological Surgery Neuroimmunology	Rush University Chicago, Illinois	Voting Member
*William Duffell, Jr. Ph.D. Government Affairs	Gambro BCT Lakewood, Colorado	Industry Representative
** Velia Butcher, J.D. Community Outreach	Water for Children of Africa San Diego, California	Consumer Representative
Joseph Parisi, M.D. Neuropathology	Mayo Clinic Rochester, Minnesota	Deputized Voting Member
Avindra Nath, M.D. Ph.D. Neurology	Johns Hopkins University Baltimore, Maryland	Deputized Voting Member
Oscar L. Lopez, M.D. Neuropsychology	University of Pittsburgh Pittsburgh, Pennsylvania	Deputized Voting Member
***Brent Blumenstein, PhD. Statistician	TriArc Consulting Firm Seattle, Washington	Deputized Voting Member

* Dr. Duffell is the Industry Representative on the Hematology Devices Panel.

** Velia Butcher is the Consumer Representative on the Molecular and Clinical Genetics Panel.

*** Dr. Blumenstein is a voting member on the General and Plastic Surgery Devices Panel.

Contents

This panel package presents information associated with PMA submission P040010 by Nymox Corporation (the sponsor), as reviewed by the Division of Immunology and Hematology Devices (FDA). It is for use in support of presentations to be made before the Immunology Devices Panel on 15 July 2005.

There are two segments in the packet, one prepared by the sponsor and one by the FDA pre-market review team. Orientation to comments by the sponsor is provided in an executive summary to that section at the front of Tab 10.

Currently proposed labeling for the device is included at page 34 in the Sponsor's Briefing Document.

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TAB 1

FDA Executive Summary

FDA Executive Summary

Introduction

Alzheimer's Disease (AD) causes high public health concern because of its increasing prevalence, the difficulty of establishing an AD diagnosis early and/or with certainty, and the limited opportunities at present for intervention. The sponsor's product, the Alzheimer Alert NTP Test (AD7C – Urine NTP Test) for neural thread protein in urine, aims at one segment of the AD puzzle – diagnosis via measures short of brain biopsy or autopsy.

The PMA submission presents analytical performance data for the NTP test and compares results from NTP testing with results from the currently used diagnostic standard of care which is based largely on criteria for probable and possible AD published by the National Institute of Neurological and Communicative Disease and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) and for Mild Cognitive Impairment (MCI) published by the Quality Standards Subcommittee of the American Academy of Neurology.

The sponsor states “the utility of the NTP level is to help the physician in the decision to pursue further diagnostic evaluation for cases of suspected AD, including specialist referral. As an adjunct, the urine NTP is a part of the overall puzzle in the evaluation of a case of suspected AD.”
(Amendment 006, 29 April 2005)

FDA review of the submission has focused on the intended use population and setting, pre-analytical issues and analytical validity, clinical validity and clinical utility.

Intended Use Population and Setting

The original study protocol developed by the sponsor identified as the intended use population, patients with suspected dementia not yet diagnosed with AD. This protocol also indicated that complete sets of clinical and ancillary data suitable for use in the differential diagnosis of AD using the diagnostic algorithm of the Quality Standards Subcommittee of the American Academy of Neurology would be provided. It was expected that a study of this population would produce data to show that positive NTP

results would warrant additional testing for AD and negative NTP results would warrant additional testing for non-AD causes of dementia.

The final data set provided to FDA included patients who were enrolled at various stages of AD work-up. There was no clear or consistent relationship between the date of enrollment and formulation or execution of work-up decisions for each patient. In response to requests from FDA, the sponsor subsequently provided additional laboratory results, and particularly additional brain imaging results. (See the Sponsor's Briefing Document.)

Clinical diagnoses in this submission are provided by the sponsor based on expert neurologist review of the cases studied. In the most recent communication (page 3, Tab 10 of this packet), an "Anticipated Use" is put forth "as an early aid to diagnosis, to be used by the non-specialist physician to help in the decision to proceed to further diagnostic evaluation, including referral and more testing."

Pre-analytical Issues and Analytical Validation

About one-third of the patients initially enrolled in the study did not provide a sample suitable for analysis. The effect of these exclusions on test performance, if any, is not clear.

Within-patient variability in NTP results indicated changes of 5 µg/mL or more, in some individuals when studied over a one month period (Amendment 006, 29 April 2005, reproduced as Appendix 1 at the end of this packet Tab). Analytical variation at laboratory sites other than that of the sponsor showed intra-run variability with a standard deviation of 2 to 3 µg/mL. Given the fact that 23% (46/200) of patients have values within 2 µg/mL of the cut-off of 22 µg/mL, the impact of these variations on clinical performance is of concern (see below). This performance appears to have been optimized by limiting analysis to samples from first morning voids and with appropriate specific gravity.

Clinical Validity

Clinical validity issues for the NTP test relate to the manner and degree to which NTP measurements reflect the patient's state, i.e. presence or absence

of AD. The sponsor's study probed the correlation of NTP level (a quantitative variable, studied both in terms of a cut-off value and via a receiver operating characteristic (ROC) analysis) with a continuum of four clinical diagnoses – definite non-Alzheimer's Disease (def non-AD), Mild Cognitive Impairment (MCI), possible AD and probable AD.

Table 1

	Intended Use Population				
	Prob AD	Poss AD	MCI	Def Non-AD	
NTP > 22	51	21	22	4	98
NTP ≤ 22	6	35	21	40	102
	57	56	43	44	200

The core analysis contrasts NTP values for patients at the extremes of that continuum, i.e. def non-AD vs probable AD. (See Table 1.) This analysis does not account for possible spectrum bias, in that probable AD patients and def non-AD patients may not include “difficult” or complex diagnostic cases (for which an unknown number may have been assigned to the possible AD or MCI categories that comprise about 50% of the study set).

The agreement of positive NTP values with probable AD (so-called sensitivity) was $51/57 = 89.5\%$, 95% CI: 78.5% to 96.0%. However, positive NTP values were also identified in 38% (21/56) of patients with possible AD, 51% (22/43) of patients with MCI, and 9% (4/44) of patients with def non-AD.

In evaluating all patients with probable AD (as noted in our memo of March 10, 2005), 89% (51/57) of patients with probable AD will have elevated NTP. However, 33% (47/143) without probable AD will also have elevations. As a result 48% (47/98) of patients with elevated NTP values will be classified as probable AD but will not have this diagnosis.

The agreement of negative NTP values with def non-AD (so-called specificity) was 90.9% (40/44), 95% CI: 78.3% to 97.5%. However, negative NTP values were also identified in 49% (21/43) of patients with MCI, 63% (35/56) of patients with possible AD, and 11% (6/57) of patients with probable AD.

In evaluating all patients with def non-AD (as noted in our memo of March 10, 2005), 91% (40/44) of patients with def non-AD have normal levels of NTP. However, 40% (62/156) of patients who are not classified as def non-AD have normal levels of NTP.

As a result 60% (62/102) of patients with normal NTP values will be classified as def non-AD but will actually have MCI, possible AD, or probable AD.

Table 2

	Not Def Non-AD	Def Non- AD	Total
NTP > 22	94	4	98
NTP ≤ 22	62	40	102
Total	156	44	200

The sponsor (Table 2) has identified as positive anything that is not def non-AD. The positive predictive value for that determination is 95.9% (94/98) with a 95% confidence interval (95% CI) of 90.0% to 98.4%. This positive result does not discriminate between patients with MCI, possible AD and probable AD. If the diagnosis of def non-AD is ruled out on the basis of a positive NTP result, an incorrect decision can occur in up to 10% of tested patients (see CI above.) Note that when the NTP result is negative, 61% (62/102) of patients will be incorrectly categorized as having def non-AD.

Table 3

	Prob AD	Not Prob AD	Total
NTP > 22	51	47	98
NTP ≤ 22	6	96	102
Total	57	143	200

The sponsor (Table 3) has identified as negative anything that is not probable AD. The negative predictive value for this determination is 94.1% (96/102) with a CI of 87.8% - 97.3%. This negative result does not

discriminate between patients with possible AD, MCI and def non-AD. If the diagnosis of probable AD is ruled out on the basis of a negative NTP result, an incorrect decision can occur in up to 12.2% of tested patients (see CI above). Note that when the NTP result is positive, 48% (47/98) of patients will be incorrectly categorized as probable AD.

Two other issues decrease confidence in the clinical test performance as presented in the submission. First, imprecision of the NTP assay near the cut-off point increases uncertainty as to how any particular patient might be classified in repeated measures. In the reported study, 23% (46/200) of the patients had NTP results in the range 20 µg/mL to 24 µg/mL, i.e. within the range spanning the cut-off value +/- one standard deviation of about 2 to 3 µg/mL that was reported for intra-run variability at three sites excluding the sponsor's laboratory. There is, in addition, uncertainty concerning accurate classification using the NINCDS/ADRDA criteria, as to the presence of histologically demonstrable AD. In two studies^{1,2}, classification as probable AD was histologically matched in about 90% of cases, and possible AD and non-AD classifications were reported to be less reliable.

Clinical Utility or Effectiveness

Given the significant overlap observed in NTP values between the four categories of interest (probable AD, possible AD, MCI, and def non-AD), it is unclear if this device is effective at meeting the stated indications for use³:

1) to refine the physician's decisions concerning additional patient evaluation for AD through "more in-depth, costly, and time-consuming diagnostic procedures..." or through "further diagnostic workup (such as specialist consultations, imaging, in-depth neuropsychological testing, EEG and other testing procedures)...", and

2) to "add more certainty for the confirmation a probable Alzheimer's disease cause of cognitive or memory disorder or dementia symptoms."

¹ Galasko D, Hansen LA, Katzman R, Wiederholt W, Masliah E, Terry R, Hill LR, Lessin P, Thal LJ. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. *Arch Neurol.* 1994;51:888-895.

² Blacker D, Albert MS, Bassett SS, Go RCP, Harrell LE, Folstein MF. Reliability and validity of NINCDS-ADRDA criteria for Alzheimer's disease. *Arch Neurol.* 1994;51:1198-1204.

³ The sponsor has submitted several versions of Intended Use and/or Indications for Use. These are reproduced fully in the FDA Statistical Review at Tab 7 of this packet.

For the first intended use, can a negative test be reliably used to rule out AD and the need for further evaluation of this disease? Can a positive test be reliably used as a basis for further AD testing? Do the data presented support this decision making?

For the second intended use, how will a positive or negative NTP value be used to increase certainty in the clinical impression?

The essential difficulty with an “added certainty” claim in AD diagnosis is that it requires comparison of diagnoses, with and without access to NTP data, to a higher diagnostic truth. In the absence of such a comparison, it is unclear, when NTP results agree or disagree with the diagnostic criteria used, whether diagnostic performance has been changed. Hence, any assertion of changed accuracy cannot be established.

Appendix 1

Table C: Urine Samples On >1 Different Days in the Same Individual

No.	Age	Sex	NTP ($\mu\text{g/mL}$)	Interval (I = initial)
1	41	F	13	I
			18.4	1 mo.
			16.4	2 mos.
2	79	F	22	I
			29.1	1 mo.
3	79	M	37.6	I
			43.5	1 mo.
4	101	F	42.4	I
			47.1	2 days
5	91	F	>60	I
			53.2	1 mo.
			56.3	1 mo.
			>60	2 mos.
			54.4	2 mos.
			>60	3 mos.
6	3	M	<8	I
			21.5	1 yr.
7	77	F	20.7	I
			10.1	1 yr.
			11	2 yr.
			19	2 yr.
8	81	M	22	I
			29.7	1 mo.
			46.	2 yr.
9	71	M	>60	I
			45	8 mos.
			39.4	8 mos.
10*	78	M	<8	I
			15.3	1 mo.
			29*	3 yr.
			46*	4 1/2 yr.
11	45	M	<8	I
			13.4	1 yr.
12	81	F	>60	I
			43.8	2 weeks
			30.9	3 mos.
			32.8	3 mos.
13	42	F	17.7	I
			14.9	2 mos.
14	38	M	11.3	I
			12.3	1 week
			11	4 mos.
			11.6	5 mos.

* Clinical diagnosis of AD 3 years after initial measurement.
Patient asymptomatic at initial measurement.

TAB 2

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TAB 3

Sponsor's Summary of Safety and Effectiveness

III. SUMMARY OF SAFETY AND EFFECTIVENESS DATA

III. SUMMARY OF SAFETY AND EFFECTIVENESS DATA

III.A Indications for Use

The Urine NTP Test is a laboratory assay intended to provide adjunctive diagnostic information to be used in the assessment of patients with signs and symptoms of possible cognitive or memory disorder or possible dementia. Test results, when considered in conjunction with other laboratory tests (of exclusion), e.g. CBC, routine serology, thyroid function tests, tests for syphilis, etc., will add more certainty in the decision to proceed to, or to avoid, more in-depth, costly, and time-consuming diagnostic procedures for the confirmation or exclusion of neurodegenerative disease causes of cognitive or memory disorder or dementia symptoms.

III.B Device Description

III.B.1 Generic Names of the Device

Urinary Neural Thread Protein Test (Urine NTP Test)

III.B.2 Trade Names of the Device

AlzheimAlert™ Test; AD7C-NTP™ Test

III.B.3 How the Device Functions

The assay is a competitive affinity assay that detects NTP. A microtiter plate is coated with a specific receptor that has a high affinity for both the Fc portion of rabbit IgG and NTP. NTP in patient urine sample or standard competes with an alkaline phosphatase (AP) labeled rabbit IgG for binding. In the absence of NTP, the plate binds the AP conjugate and absorbance is high. In the presence of NTP, competitor binding is decreased in proportion to the amount of NTP present.

III.B.4 Basic Scientific Concepts Underlying the Device

NTP has been found to be a protein expressed in brain, and there is evidence that it is involved in neuronal sprouting, apoptosis, and cell death. It also is a marker for Alzheimer's disease (12-53). Alzheimer's disease is a presently incurable neurodegenerative disease affecting 10-15 million people worldwide. It is characterized pathologically by many abnormalities, the most significant of which are senile plaques, neurofibrillary tangles (NFT), brain cell loss and cerebral atrophy.

Neural thread proteins (NTP) are a novel family of recently characterized brain proteins. AD7C-NTP is a ~41 kD membrane associated phosphoprotein with functions related to neuritic sprouting and cell death (12-14, 20, 22-24). AD7C-NTP mRNA is upregulated in Alzheimer's disease brain compared to controls. AD7C-NTP protein levels in brain and in CSF are higher in Alzheimer's disease compared to controls, and

AD7C-NTP immunoreactivity is clearly found in senile plaques, in NFT, in degenerating neurons, neuropil threads, and dystrophic neuritic sprouts in Alzheimer's disease and Down syndrome brains (12-25). AD7C-NTP accumulation in neurons occurs early in Alzheimer's disease neurodegeneration (before NFT formation), and neuronal cells in culture transfected with the AD7C-NTP gene exhibit cell death with neuritic sprouting (12, 20, 24).

III.C Alternative Practices and Procedures

The process of clinical diagnosis of dementia, and the diagnosis of the underlying disease(s) causing the dementia, are currently imperfect and fraught with difficulties. Much of the problem is attributable to the following factors: (1) disease expression variability (cognitive and emotional symptoms, individual personality variation, etc.) (2) disease chronicity (symptom duration of 7-10 years or more); (3) doctor and other health care provider training variability (general practitioners, psychiatrists, neurologists, geriatricians, psychologists, nurses, social workers, assistants); (4) office or institutional facilities variability (presence or absence of subspecialists, state-of-the-art scanners, MRI, etc.); (5) mixed pathologies (symptoms produced by more than one causal pathology, e.g. Alzheimer's disease plus stroke, etc.); (6) lack of standardization (of stage of work-up, of criteria, of ancillary procedures, etc.) The end results are: (1) inaccurate or uncertain diagnosis, and (2) delayed diagnosis which in turn may cause delayed treatment, (3) patient

and family uncertainty and anguish, (4) poor planning for patients and families (for care, for estate) and other problems. An already tragic situation is thus further complicated. In most situations of clinical dementia, there is significant caregiver morbidity, with serious emotional and financial consequences, also accentuated by imperfections in the diagnostic process.

The diagnostic work-up of a case of suspected dementia will therefore vary according to (1) the patient's state of illness, (2) the expertise and training of the doctors treating the patient, (3) the institutions and facilities involved. Standard textbooks and references reflect this latitude.

The algorithm based on recommendations published by the Quality Standards Subcommittee of the American Academy of Neurology (1994, 1997, 2001) (Appendix 2) is representative of current medical practice. Subject to the clinical variability of disease presentation and progression and availability of resources, there is an orderly progression from history and physical (Standards), to possible initial laboratory testing (blood screening, etc.) (Guidelines) to possible in-depth special testing procedures (e.g. lumbar puncture, PET, SPECT, MRI, EEG, etc.) (Options).

The current procedures, taken together, minimize the error given current states of knowledge. Nevertheless, none of the procedures taken alone is a stand-alone diagnostic. The diagnosis is generally a diagnosis of exclusion. The main shortcomings of the process are that it is uncertain,

inefficient, costly, and slow. A test that can add certainty to the process will be useful. A test that can streamline the flow chart will increase efficiency and speed, and will also reduce cost to the system.

The diagnosis of dementia and Alzheimer's disease is a complex process which is often lengthy and inefficient. Physicians vary greatly in training, experience, and expertise in dementia diagnosis. Time duration from the initial symptoms to the physician's diagnosis of Alzheimer's disease averages approximately 33 months (2). Definitive diagnosis requires brain biopsy or postmortem histopathological studies (3). Antemortem clinical diagnosis can be up to 80% or more accurate depending upon the extent of specialized investigations, the experience and expertise of the clinical center and clinicians carrying out the investigations and follow-up (4-11). For example, the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology has considered single photon emission computed tomography (SPECT) (which for Alzheimer's disease has a sensitivity of 35-45% and a specificity of 90%) an established technique to support the clinical diagnosis of Alzheimer's disease (4). Positive predictive value for the diagnosis of Alzheimer's disease in the average clinical situation is in the 50-70% range (5-11). In the initial work-up of a case of suspected dementia, a number of diagnostic and laboratory procedures are carried out, which if normal (negative) may or may not lead to more involved and costly diagnostic procedures, for example, imaging studies such as computerized tomography (CT), positron emission tomography (PET), magnetic

resonance imaging (MRI), single photon emission computerized tomography (SPECT), electroencephalography (EEG), lumbar punctures, and biopsies. These more specialized complex procedures increase the diagnostic accuracy. A positive or negative indicator which can, at the stage of the initial laboratory work-up, help the clinician distinguish probable AD from non-AD controls will be of significant clinical utility.

FDA Approved Diagnostic Devices for AD/Dementia

There is no previously approved diagnostic device for AD or dementia.

Imaging Methods for the Diagnosis of AD and Dementia

CT, PET, MRI, SPECT and other methods are legal devices used for neurodiagnostic indications. These modalities provide reliable diagnostic information for a wide range of conditions such as tumor, ischemic or hemorrhagic stroke, trauma, hydrocephalus, etc., and thus validly contribute to the diagnostic workup of neurological, geriatric, and psychiatric conditions by helping to rule out those conditions if they are suspected. The utility of imaging methods for the diagnosis of dementia is still under investigation.

III.D Marketing History

The urinary neural thread protein test kit has not been marketed previously as a kit:

The urinary neural thread protein test in its present format has been marketed as a CLIA certified test by the sponsor since 2000. The test has been available from the sponsor's CLIA certified clinical reference laboratory (Maywood, New Jersey).

III.E Summary of Studies

III.E.1 Summary of Results of Non-Clinical Laboratory Studies

III.E.1.i Drug Interference Studies

Low, medium, and high-NTP urines were tested before and after spiking with the following drugs (25 µg/mL): tetracycline, atenolol, digoxin, diltiazem, captopril, coumadin, prozac, glyburide, meperidine, metoprolol, nifedipine, l-thyroxine, hydrochlorothiazide, furosemide, codeine, cephalexin, alprazolam, lorazepam, flurazepam, norvasc, vasotec, metformin, amoxicillin, sertraline, biacin, fosamax, lipitor, losec, acetaminophen, ibuprofen, potassium, pepcid, indur, temazepam.

None of the spiked drugs had significant effect on the NTP urine test values of any of the urine samples.

III.E.1.ii Other interference studies

Low, medium, and high-NTP urines were tested before and after spiking with the following substances: human gamma globulin (100 µg/mL), acid -1-glycoprotein (100 µg/mL), human serum albumin (100 µg/mL), bilirubin (20 µg/mL), fresh blood (250 RBC/mL), hemolyzed blood (250 RBC/mL), gram negative bacteria, and lipids (1.5 mg/mL) (cholesterol and lipoprotein (C-5555, Sigma)).

None of the above spiked substances had significant effect on the NTP urine test values of any of the urine samples.

III.E.1.iii Urine concentration studies

High-NTP urine was spiked into low-NTP urine where the latter, before spiking, was concentrated at 5 different levels (5%, 10%, 15%, 20%, and 25%), and the spiked variably concentrated urines were then assayed (5 levels of urine concentration for each of 5 levels of NTP concentration (95 µg/mL, 62.2 µg/mL, 29.4 µg/mL, 24.7 µg/mL, 20 µg/mL).

There was no significant effect of concentration of urine on the NTP urine test values.

III.E.1.iv Recovery studies

Low NTP urine samples were spiked with known concentrations of NTP to 18.9 µg/mL, 23.9 µg/mL, 28.9 µg/mL, 33.9 µg/mL, and 38.9 µg/mL, and 20 replicates of each were assayed.

The mean recovery from the 120 replicates was 105.5%.

III.E.1.v Analytical Threshold

The limit of detection (at 405 nm) is OD 1.315 ± 0.8 , corresponding to a threshold of 10 µg/mL.

III.E.1.vi Precision Studies

A total of 720 replicates were assayed at 4 different clinical laboratory sites by 4 different trained laboratory personnel, on 3 different days each, consisting of high, medium, and low urines in 20 replicates each per day.

The CVs varied from 2.3% to 7.1% (high-NTP urines), 1.5% to 8.5% (medium-NTP Urine), and 2.5% to 15% (low-NTP urine).

III.E.1.vii Stability Studies

Stability studies indicate that refrigerated coated plates are stable for 10 weeks. Standard is stable at 4°C for ≥ 6 months. Urine controls are stable at -20°C for 3 months. Other NTP test kit components have

stability \geq 3 months. Urine NTP test kits must not be used later than the kit label expiry date.

III.E.1.viii Freeze-Thaw of Processed Urine

Fresh unprocessed urine should not be frozen prior to assay in the urine NTP test. Nonspecific protein aggregation may occur which can alter the assay. Processed urine samples (high, medium, and low-NTP urine) were assayed, frozen at -80°C , and then tested (one freeze-thaw) in 135 replicates on 45 different days up to 67 days. The CVs of freeze-thawing of processed urine over time were 5.6% (high NTP urine); 11.8% (medium-NTP urine); and 9.8% (low-NTP urine).

III.E.1.ix Transport of Kit

Urine NTP test kits in styrofoam boxes (frozen test kit components shipped with dry ice) were shipped coast to coast in the U.S. over a 3 day interval. Kits (standards and control urines) were tested before and after shipment.

There was no significant effect of transport on NTP urine test results.

III.E.2 Summary of Clinical Investigations

A prospective study was conducted to enroll patients undergoing workups who have symptoms or complaints of cognitive problems or dementia. A

total of 200 patients were studied. All enrolled and consented patients had urinary samples collected at the onset of the evaluation period, and all subsequently went through extensive neurological and physical testing. The results of the NTP assay were not made available to the investigators, patients, or any of the physicians involved in the clinical assessment of the subjects. Each of the subjects was assigned to a diagnosis category based on the opinions of the investigating physicians, who used the results of a variety of tests to make a decision on each patient. The four possible categories, which directly reflect the state of the art in current neurological workups, were as follows:

Category 1 – Probable AD

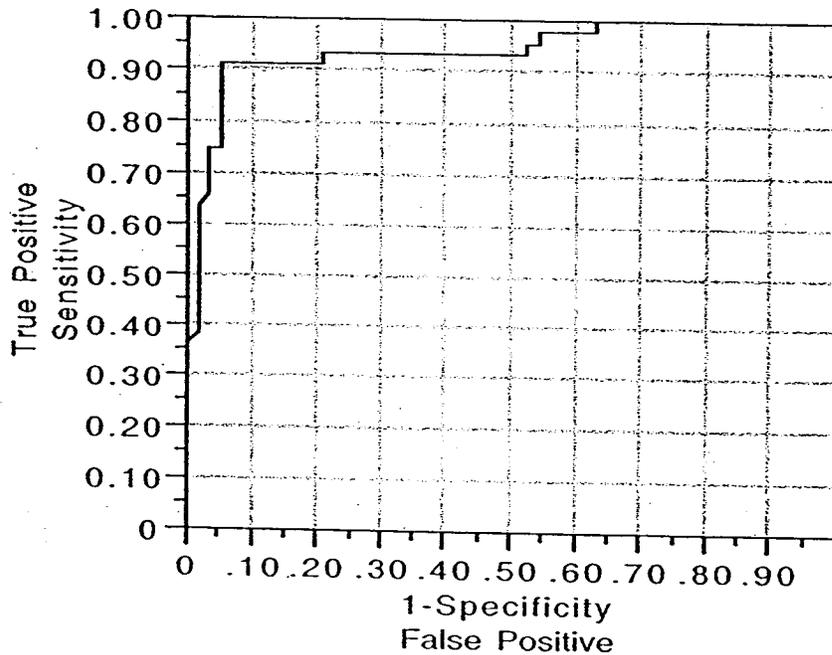
Category 2 – Possible AD, including cases with mixed multiple syndromes

Category 3 – Mild cognitive impairment (MCI), which may be a precursor to AD development

Category 4 – Definite non-AD, with a diagnosis confirmed by psychological or physiological tests

Figure III.E.2.1 shows the ROC curve of Probable AD (Category 1) versus Definite non-AD (Category 4). The ROC curve exhibits a healthy 0.94 area under the curve (AUC), and for AD vs. MCI, the AUC was 0.73, both of which reinforce the clinical utility of the test in differentiating these pairs of groups.

Figure III.E.2.1
ROC Curve
Discrimination prognosis: Probable AD vs. Non-AD



Area under curve (AUC) = 0.9398

Throughout these analyses, "RISK" is used as the mode of measurement of the utility of the NTP test. This form of risk is not that of eventually developing the disease, such as is used for evaluating cholesterol levels as a "risk" factor in coronary heart disease. For this disease entity, risk refers to the probability that the patient already has the disease.

The results of the logistic regression analysis of the odds ratios show that urinary NTP is a very effective tool for assessing risk of AD. While there is some overlap in NTP results between diagnostic categories, there is a strong tendency for NTP to be elevated in cases of probable Alzheimer's disease, compared to NTP levels in the possible, mixed, and non-AD groups. The results of the study analyses indicate that risk rises as NTP increases, and that thresholds of both 20 and 30 provide useful prognostic assistance to the neurological workup process.

The Kruskal-Wallis test shows a statistically significant ($p < 0.0001$) difference in the mean result between the groups, and in fact the scatterplot demonstrates the fact that the mean NTP result is largely monotonically decreasing with decreasing likelihood of AD, even though there is considerable overlap in the NTP distributions between adjacent groups.

The probable AD subjects tended to be slightly older than the other 3 categories, with a significant p-value for both the ANOVA and the Kruskal-Wallis analysis (both < 0.0001). In fact the ages show a small but monotonic decrease in age with decreasing AD likelihood, which matches clinical practice. In some cases this would require a covariate adjustment for age in the assessment of risk, however the NTP results are not correlated with age, so such adjustment was not deemed necessary for this study.

There were no significant differences noted in gender split between the 4 diagnostics groups ($p=0.47$ by chi-squared contingency table analysis).

The odds ratio calculations show the splits between NTP distributions for AD and non-AD groups, using 20 and 30 as the decision threshold. In each case, the odds ratios are computed along with the (asymptotic) 95% confidence intervals. In both cases, the confidence intervals lie well above 1.00, indicating the statistically and clinically significant increase in odds when the NTP assay result lies above the indicated threshold. For the threshold of 20 ug/mL, the odds ratio is 47.4, and at 30 it is 21.3, both significantly higher than 1.00, and both fairly dramatic indications of the utility of the test in risk assessment.

III.F Abstracts of Conclusions Drawn from the Studies

III.F.1 Discussion of valid scientific evidence

The primary study hypothesis was that the results of the urinary NTP assay provide a statistically significant ability to assign risk categories for probable AD against those who are found to be definitely non-AD. Secondary hypotheses were that the probable AD group could be differentiated against the other two categories of possible AD (including mixed cases and multiple dementia syndromes), and the category of mild cognitive impairment (MCI).

The primary statistical tool employed to test these hypotheses was the use of nominal logistic regression and computation of the odds ratio along with its 95% confidence interval. The results of the logistic regression analysis of the odds ratios show that urinary NTP is a very effective tool for assessing risk of AD. While there is some overlap in NTP results between diagnostic categories, there is a strong tendency for NTP to be elevated in cases of probable Alzheimer's disease, compared to NTP levels in the possible, mixed, and non-AD groups.

The ROC curve for probable AD vs. non-AD exhibits a healthy 0.94 area under the curve (AUC), and for AD vs. MCI, the AUC was 0.73, both of which reinforce the clinical utility of the test in differentiating these pairs of groups. The results of the study analyses indicate that risk rises as NTP increases, and that thresholds of 20, 22 and 30 provide useful prognostic assistance to the neurological workup process.

III.F.2 Discussion of data on safety and effectiveness

Safety: The investigation involved a voided urine sample being assessed as a laboratory adjunctive aid to diagnosis. The investigation posed no increased risk to the subjects.

Effectiveness: The results of the study analyses indicate that risk rises as NTP increases, and that thresholds of 20, 22, and 30 provide useful prognostic assistance to the neurological workup process. The ROC curve for probable AD vs. non-AD has a 0.94 area under the curve (AUC),

and for AD vs. MCI, the AUC was 0.73, both of which reinforce the clinical utility of the test in differentiating these pairs of groups.

III.F.3 Risk/benefit analysis

The NTP urine test essentially poses no risk, involving a voided urine sample being assessed as a laboratory adjunctive aid to diagnosis.

Benefits: A prospective blinded clinical study was conducted to evaluate the clinical utility of urinary NTP as an aid in the neurological and physiological diagnosis of Alzheimer's disease. Differentiation of Alzheimer's from other forms of dementia is difficult. Because of the enormous social and psychological effect of this disease, any physiological markers which can assist in the differential diagnosis and risk assessment process would have immediate clinical utility, and even more so as treatment options are developed.

The ROC curve for probable AD vs. non-AD has a 0.94 area under the curve (AUC), and for AD vs. MCI, the AUC was 0.73, both of which demonstrate significant clinical utility of the test in differentiating these pairs of groups.

Odds ratio calculations indicate the statistically and clinically significant increase in odds when the NTP assay result lies above the indicated threshold. For the threshold of 20 µg/mL, the odds ratio is 47.4, and at 30

it is 21.3, both significantly higher than 1.00, and both fairly dramatic indications of the utility of the test in risk assessment.