

TAB 4

FDA issues, July 2004

FDA sent a list of review issues to the sponsor on 22 July 2004. These issues were described as follows:

- There was no consistent or reliable identification of patients with or without probable or possible Alzheimer's disease.
 - No protocol appeared to be followed or to be consistently in place to ensure a reliable accrual of diagnostic information on neurological exams, radiographic exams, or blood work needed to establish reliable characterization of patients using standard diagnostic criteria.
 - In particular data accrual failed to take into account the temporal relationship between final diagnoses presented in the PMA and supporting clinical evaluations, neurological studies and/or blood work.
 - In numerous cases diagnostic information was temporally unrelated (by months to years) to the performance of the urine NTP test. In numerous additional cases, insufficient data (often constituting little more than truncated and minimal neurological assessment) was presented on case report forms to demonstrate how diagnostic categorization was made.
 - It is unclear how many patients being studied in this submission entered the study as undiagnosed and newly referred patients according to the study protocol.
-
- The data you provided cannot be evaluated because there appear to be no clear and consistent pre-screening of urines prior to NTP testing to ensure lack of interfering substances and proper sample integrity.
 - Please note that because of the uncertainty in the definition of the patient populations studied, in the selection criteria used in patient recruitment, and in the integrity of the urine samples subject to analysis, an in depth statistical review of this submission was not possible.

TAB 5

FDA issues, March 2005

FDA sent a list of review issues to Sponsor on 10 March 2005. These issues were described as follows:

We have used the entire data set and evaluated this with the recommended Nymox cut-off point of 22 µg/mL for neural thread protein (NTP) in the table below.

Cutoff = 22µg/mL

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

We agree with Nymox that 89% (51/57) of patients with Probable Alzheimer's Disease (AD) have elevated NTP. However, 33% $((21+22+4) / (56+43+44) = 47/143)$ of patients without Probable AD also have elevated NTP. As a result 48% (47/98) of patients with elevated NTP values will be classified as Probable AD but will not have this diagnosis.

We also agree with Nymox that 91% (40/44) of patients with Definite Non-AD have normal levels of NTP. However, 40% $((6+35+21) / (57+56+43) = 62/156)$ of patients who are not classified as Definite Non-AD have normal levels of NTP. As a result 61% (62/102) of patients with normal NTP values will be classified as Definite Non-AD but will actually have Mild Cognitive Impairment (MCI), Possible AD, or Probable AD.

Clinical Study Concerns

1. In the most recent submission of December 3, 2004, Nymox has modified its intended use to read the NTP test would add certainty in the confirmation of probable Alzheimer's disease, i.e., after other testing has been completed.

FDA concerns about test performance are increased by the fact that the definition of the two groups of interest (Probable AD and Definite Non-AD) is based on agreement with the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria. This provides no information or insight into the added certainty contributed by the NTP result.

The ROC curve provided in your submission does demonstrate the correlation between NTP and NINCDS/ADRDA criteria. But there appears to be no higher order of truth delineating test performance. The contribution of NTP to decision making when the diagnostic criteria either agree or disagree is unknown. In short, when NTP results are added to

NINCDS/ADRDA results, it is not clear whether or how diagnostic performance has changed.

Objective proof of added certainty from NTP testing would require a comparison of diagnoses, rendered with and without NTP results, to a higher diagnostic standard.

2. The scientific endpoint chosen to characterize NTP performance was the use of diagnosis by a neurologist of Probable AD, Possible AD, MCI, and Definite Non-AD. The diagnostic algorithms used to catalogue patients were done with non-standardized and non-concurrent diagnostic testing across sites that were retrospectively completed. This approach may have created a broad and unusually heterogeneous diagnostic population.

The issues of failure to apply a well defined algorithm including clinical, radiological and laboratory findings was raised during the initial review of the March 2004 original submission. This issue remained unresolved in the material sent to FDA request in your June 21, 2004, amendment.

In October and November 2004, you forwarded additional materials including imaging studies for 18 patients who were diagnosed with Possible or Probable AD. No additional laboratory data were sent with the amendment. One-sheet case summaries were provided with supportive criteria and a list of excluded diagnoses.

Please clarify how the company has determined that the methodologies used have created a reasonable base for establishing diagnostic performance for comparison with NTP. Of note, two articles on diagnosis of Alzheimer's Disease (1, 2) have noted that clinical diagnosis when compared to either pathological diagnosis or considered in comparison to consensus neurological diagnosis had sensitivities as low as 81% and specificities as low as 73%.

3. FDA is particularly concerned that there is not an explicit basis for assigning study subjects to the Definite Non-AD group. As far as we can tell from evaluating your submission, the study patients include few or none in the Definite Non-AD group who can be credibly confused with Alzheimer's disease through application of the NINCDS/ADRDA criteria. Examples of such patients might include individuals with frontotemporal dementia, dementia with Lewy bodies and pseudodementia due to depression. Please clarify how the Definite Non-AD patients were chosen and explain why you believe NTP results in this group would be of use in the differential diagnosis of patients with various forms of dementia.
4. The use of the cut-off of 22 $\mu\text{g}/\text{mL}$ was drawn post hoc from the study itself and never independently validated. While it would be possible to apply bootstrapping or other techniques to substitute for an independent assessment of the cut-point, these statistical techniques are likely to lead to decreased estimates of performance. Please provide an estimate of the bias and performance decrement associated with use of a post hoc cut-off for the study.
5. There is little in the submission to address the possibility of matrix variability due to differences in hydration. Day-to-day biological variability in the marker has not been

characterized. Urines were screened using specific gravity and dilute samples discarded but it is not clear whether multi-day sampling or correction using creatinine might help provide more predictive results.

6. The transfer of neural thread protein from brain through the blood brain barrier to blood and urine (which has not to our knowledge undergone pharmacokinetic studies) may not be constant enough to produce a reliable signal for this type of neurological disease.
7. Please note that the intended use for the device appears to have changed from that present in your protocol submitted for pre-IDE review and in the Device Description from your original PMA submission. According to these previous submissions, the NTP test will be applied very early in the evaluation of patients who have symptoms consistent with Alzheimer's disease and will help guide their further evaluation. FDA's expectation was that patients still at an early point in their evaluation (i.e., before undergoing more in-depth, costly and time-consuming diagnostic procedures) would be enrolled. This expectation was not met, in that patients at disparate points in their evaluation and care were enrolled instead. In addition, the study did not demonstrate appropriate reliance on NTP results to avoid specific advanced diagnostic tests. If you plan to eliminate this previous claim, this does not pose a problem. However, if you do wish to include the original claim as part of your submission, please clarify how this claim would relate to the data generated by your study.
- 8.

Analytical Studies

9. The submission is unclear about the nature and derivation of critical reactants in the NTP test. Please provide data detailing the following characteristics for the PN3 protein: chemical nature, source, binding affinity for NTP as it will be found in urine and in control/calibrator preparations, binding affinity for rabbit IgG and identity of the portion of the rabbit IgG molecule that is bound. Please provide data detailing the following characteristics for NTP protein as it is expected to be present in urine: chemical nature, binding affinity for PN3, cross-reactivity for rabbit IgG. Please also provide data detailing the same characteristics for the NTP protein used in calibrators/controls. Please identify and/or define the nature of any blocking agents applied to the plate along with PN3, and their efficiency at preventing non-specific binding of rabbit IgG and NTP.
10. The disposition of reagents during the test is unclear. Please provide a list or diagram detailing the chemical test components that are bound to the plate before commencing the test. Provide a separate list or diagram of test components in solution immediately before their addition to the test plate.

11. Please provide data demonstrating absence of non-specific binding by labeled rabbit IgG to the test plate. Please also provide data demonstrating absence of non-specific binding by urine NTP and NTP calibrator/control protein to the test plate.
12. Please provide data verifying the equivalence of NTP as found in the urine to NTP as found in the cerebrospinal fluid and brain of Alzheimer's disease patients. Demonstration of immunochemical identity will be sufficient.

References cited:

1. Galasko D, Hansen LA, Katzman R, Widerholt W, Masliah E, Terry R, Hill LR, Lessin P, Thal LJ. Clinical-neuropathological correlations in Alzheimer's disease and related dementia. *Arch.Neurology*. 1994; 51:888-895
2. Blacker D, Albert MS, Bassett SS, Go RC, Harrel LE, Folstein MF. Reliability and validity of NINCDS criteria for Alzheimer's disease. *Arch.Neurology*. 1994; 51:1198-1204.

TAB 6

FDA Medical Officer's Review

MEMORANDUM

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION**

**Division of Neuropharmacological Drug Products (HFD – 120)
Center for Drug Evaluation and Research**

Date: February 6, 2005

**Subject: Nymox Urine NTP Test
PMA 040010**

**To: Head
Division of Immunology and Molecular Diagnostics
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health**

Document Type: Consult

Enclosed is the Division's response to your request

Review and Evaluation of Clinical Data

PMA	040010
Sponsor:	Nymox
Device:	Urine NTP Test
Proposed Indication:	Alzheimer's Disease
Material Submitted:	Amendment To Application/Consult
Correspondence Date:	11/30/04
Date Received By Reviewer:	1/3/05
Date Review Completed	2/6/05

1. Background

This submission, an Amendment to a Pre-Marketing Application (PMA), has been received as a part of an ongoing consultation on this application from the Division of Immunology and Molecular Diagnostics of the Center for Devices and Radiological Health.

This Amendment is the most recent of a series of submissions that followed the issuance by this Agency of a "Not-Approvable" letter on July 22, 2004, and are in response to that letter. A meeting was also held with the sponsor subsequent to the issuance of the Non-Approval letter; this meeting on September 10, 2004 was attended by representatives of this Division (). The only submission formally reviewed by me prior to the current Amendment is the original submission under this PMA; please refer to that review (completed 7/8/04) for further details.

The application itself is in reference to a urine test for Alzheimer's Disease: the test measures a putative biochemical marker for Alzheimer's Disease referred to as "neural thread protein" (NTP), a generic term, and also as AD7C-NTP.

The test is referred to in the application as the Urinary Neural Thread Protein Test and as the Urine NTP Test. The trade names for the test are stated to be as follows: AlzheimerAlert™ Test; AD7C-NTP™ Test.

A single definitive study (Protocol N600721) was submitted with the original PMA as the basis for the Intended Use of this device.

2. Contents Of Submission

As noted above, this submission is the latest of several submissions since the issuance of the Not-Approvable Letter of July 22, 2004. These submissions have been reviewed directly by the primary reviewing Division at CDRH.

The contents of the current submission are as follows

- Executive Summary (which proposes a new Intended Use, in addition to containing other items)
- Summary of statistical analysis used to support the newly proposed Intended Use (with separate full reports of these analyses appended to the submission)
- Listing containing the dates of the most recent imaging studies for all patients diagnosed to have Probable or Possible Alzheimer's Disease (except Center #6; see below)
- A listing containing the following information for all 200 study patients: date of receipt of urine sample; diagnosis; age; sex; race; and urine NTP value
- Listings of all study patients by analysis dataset: these listings indicate diagnosis and urine NTP test value
- The basis for diagnosing patients as having Probable Alzheimer's Disease, Possible Alzheimer's Disease, and Mild Cognitive Impairment in all study patients, except Center #6 (see below)
- Reports for all updated imaging studies

3. Contents Of Review

In this review, I will confine myself to the following. Those contents of this submission that have not been reviewed by me, as well as other submissions made by the sponsor since the Not-Approvable letter was issued on 7/22/04 are to be addressed directly by the primary reviewing division.

- Original Intended Use Of Device And Outline Of Definitive Clinical Study Submitted In Original PMA
- Key Text Of Non-Approval Letter
- Proposed New Intended Use
- Sponsor's Analysis To Support Current Intended Use Of Device
- Additional Analyses By Agency Statistician
- Additional Observations
- Comments

4. Original Intended Use Of Device And Outline Of Definitive Clinical Study Submitted In Original PMA

4.1 Original Intended Use

The original Intended Use of the Urine NTP assay was as part of the early assessment of patients presenting with impaired memory or other symptoms suggesting cognitive impairment to a clinician, and was to be used prior to a definite diagnosis being made. The original Intended Use statement is as follows:

"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 a neurodegenerative disease causes of cognitive or memory disorder or dementia symptoms."

The following would appear to follow from the above statement

- The Urine NTP Test is intended for use early during the diagnostic process for a subject presenting with symptoms and/or signs suggesting impaired cognition (from this reviewer's perspective, impaired memory is a form of impaired cognition)
- The Urine NTP Test results are intended to be evaluated in conjunction with laboratory tests (these are mainly, if not entirely, blood tests) commonly used in the evaluation of patients with cognitive symptoms and/or signs, to help decide whether to proceed to further diagnostic procedures [reviewer's comment: the latter procedures commonly include CT scan or MRI of the brain, but may, less frequently, entail one or more of the following procedures such as cerebrospinal fluid examination, other forms of brain imaging, or even brain biopsy]

4.2 Key Aspects Of Design Of Definitive Study

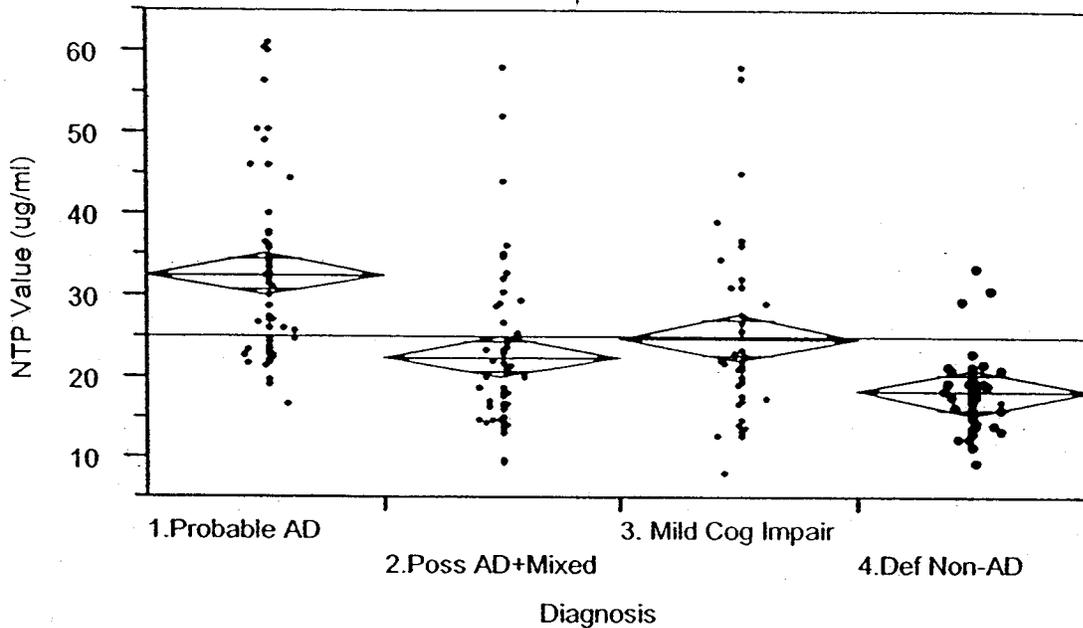
These aspects are summarized below

- Subjects included in the active study group were to be those who were newly referred, to a specialist, on account of cognitive symptoms and/or signs, prior to the diagnostic process being initiated. These subjects were to be screened through a history and physical (including neurological) examination, then provide a urine sample for NTP assay, and finally undergo a battery of assessments including Mini-Mental Status Examination, brain imaging (the need for which was to be left to the discretion of the investigator), standard laboratory tests (hematology, clinical chemistry, urinalysis), and serum Vitamin B₁₂, thyroid functions, and syphilis serology. After the evaluation was complete, they were to be assigned to one of 4 diagnostic categories: Probable Alzheimer's Disease; Possible Alzheimer's Disease; Mild Cognitive Impairment; and "Definite Non-Alzheimer's Disease."
- Healthy control subjects were also to be separately enrolled and urine NTP levels determined in that population
- The purpose of the study was to compare the measure of agreement in the designated study population between a positive Urine NTP Test and the diagnosis of either Probable or Possible Alzheimer's Disease (these diagnoses being made after an "extensive specialized work-up), and between a negative Urine NTP Test and "Definite Non-Alzheimer's Disease" or healthy control status. It was hoped that if there was a statistically significant relationship between a positive Urine NTP Test and the diagnoses of Probable or Possible Alzheimer's Disease, made after an extensive "work-up," the test could then be used along with the standard laboratory tests to help justify either pursuing or avoiding more further

investigations such as brain imaging, neurophysiological testing, lumbar puncture, and biopsy

4.3 Summary Of Key Study Results

The distribution of urine NTP results (in $\mu\text{g/mL}$) by diagnostic category is in the following figure and table, which I have copied, with very minor modifications, from the submission. The table shows the mean, standard error of mean, and 95% confidence interval for the urine NTP results for each of the 4 groups



The sponsor later clarified that the "Possible Alzheimer's Disease Plus

Diagnostic Category	Number	Mean	SEM	Lower 95%	Upper 95%
1. Probable Alzheimer's Disease	57	32.46	1.2894	29.915	35.001
2. Possible Alzheimer's Disease + Mixed	56	22.40	1.3009	19.834	24.966
3. Mild Cognitive Impairment	43	24.78	1.4846	21.854	27.709
4. "Definite Non-Alzheimer's Disease"	44	18.19	1.4676	15.299	21.088

Mixed" was no different from Possible Alzheimer's Disease

For full details of the study results, please see my original review

4.4 Key Deficiencies In Definitive Study

The following were the key deficiencies in the definitive study, based on my assessment. These deficiencies were pervasive for 6 of 9 study centers (encompassing 140/200 study subjects)

- For the majority of subjects the study report, Case Report Forms, and submitted narratives did not contain sufficient information, even when viewed in combination, to justify the assignment of each subject to one of the four main diagnostic categories (i.e., Probable Alzheimer's Disease, Possible Alzheimer's Disease, Mild Cognitive Impairment, and "Definite Non-Alzheimer's Disease"), or

to specific entities subsumed under the "Definite Non-Alzheimer's Disease" category.

- In addition,
 - The assignment of subjects to the four specific diagnostic categories often appeared to lack either a reasonable degree of uniformity and/or the correct application of protocol-specified (and standard) diagnostic criteria.
 - The assignment of subjects to specific diagnostic categories was even more questionable because the majority of subjects failed to have one or more of the following procedures performed concurrently with the study.

Brain imaging

Appropriate blood tests

Neurological examinations

Adequate cognitive testing

- A significant proportion of study subjects ($\geq 20\%$) appeared not to have been undiagnosed and/or newly referred; they had been previously evaluated at the same study center for similar symptoms and a diagnosis made. In contrast, the intended use of the Urine NTP Test was ostensibly for patients in whom a diagnosis has not previously been made.
- There were many inconsistencies in entries between the study report, Case Report Form, and narratives for individual patients. In a number of instances, tests that had been recorded in the study report as having been done did not appear to have been performed.
- Formal reports had not provided for the brain imaging procedures and/or blood tests that were performed in a number of subjects.

4.5 Reviewer's Conclusion And Recommendation

This reviewer made the following conclusion

The deficiencies in this study are so pervasive that, should the sponsor wish to pursue the development of the Urine NTP Test for the same (or a similar) indication, an entirely new study should be performed.

5. Key Text Of Non-Approval Letter

The following is the key text contained in the Non-Approval letter dated July 22, 2004

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA). We regret to inform you that CDRH has determined that your PMA is not approvable based on the requirements of 21 CFR 814.44(f), and, where practical, FDA must identify measures necessary to make the PMA approvable. Accordingly, to place your PMA in approvable form, you must provide data from a study which supports the proposed intended use and the study hypothesis that the test method had a useful percentage rate of agreement with specialized clinical and radiological investigations that can help distinguish Probable or Possible Alzheimer's Disease from non-Alzheimer's Disease individuals.

The data provided in this PMA failed to support this hypothesis because:

- There was no consistent or reliable identification of patients with or without probable or possible Alzheimer's disease.
 - No protocol appeared to be followed or to be consistently in place to ensure a reliable accrual of diagnostic information on neurological exams, radiographic exams, or blood work needed to establish reliable characterization of patients using standard diagnostic criteria.
 - In particular data accrual failed to take into account the temporal relationship between final diagnoses presented in the PMA and supporting clinical evaluations, neurological studies and/or blood work.
 - In numerous cases diagnostic information was temporally unrelated (by months to years) to the performance of the urine NTP test. In numerous additional cases, insufficient data (often constituting little more than truncated and minimal neurological assessment) was presented on case report forms to demonstrate how diagnostic categorization was made.
-
- The data you provided cannot be evaluated because there appear to be no clear and consistent pre-screening of urines prior to NTP testing to ensure lack of interfering substances and proper sample integrity.
 - Please note that because of the uncertainty in the definition of the patient populations studied, in the selection criteria used in patient recruitment, and in the integrity of the urine samples subject to analysis, an in depth statistical review of this submission was not possible.

6. Proposed New Intended Use

In the current submission, the sponsor has changed the Intended Use of this device. The text of the currently proposed Intended Use is as follows:

"The intended use for the Urine NTP Test is to provide diagnostic information to be used in the assessment of patients with signs and symptoms of dementia. Test results, when considered in

conjunction with other tests will add more certainty for the confirmation of a probable Alzheimer's disease cause of cognitive or memory disorder or dementia symptoms. The urine NTP test is not a stand-alone diagnostic and should not be interpreted without history, physical and neurological examination, and all available medical and laboratory data."

7. Sponsor's Analysis To Support Current Intended Use Of Device

These analyses are summarized below. The following summary is derived both from the submission and from the memo provided by Dr Marina Kondratovich, Statistical Reviewer, dated January 24, 2005

7.1 Analyses

- The sponsor has performed analyses that are intended to evaluate the ability of the Nymox Urine NTP test to distinguish between those assigned to the probable Alzheimer's Disease category and those in the "Definite Non-Alzheimer's Disease" category. The following having been calculated from each analysis
 - The percentage agreement of the test results with the diagnosis of probable Alzheimer's Disease and "Definite Non-Alzheimer's Disease"
 - The odds ratio resulting from the above with 95% confidence intervals
 - Areas under receiver operating characteristics (ROC) curves

- A cut-off of 22 µg/mL has been used in these analyses with values > 22 µg/mL being considered positive and those equal to or less than 22 µg/mL being considered negative

- 3 study populations have been subjected to the same analyses
 - All 200 patients enrolled in the study
 - A subset consisting of 168 out of 200 patients enrolled, with 32 patients at a study site in Miami Beach, Florida, being excluded
 - A subset consisting of 178 out of 200 patients enrolled, with 22 patients being excluded as they were not newly referred for evaluation

The analyses for all 3 of the above populations is summarized below

7.1.1 All 200 patients

	Probable Alzheimer's Disease	"Definite Non-Alzheimer's Disease"	Subtotals
NTP > 22	51	4	55
NTP ≤ 22	6	40	46
Subtotals	57	44	101

Agreement of Probable Alzheimer's Disease with positive NTP test = 89.5% (51/57)
 Agreement of "Definite Non-Alzheimer's Disease" with negative NTP test = 90.9% (40/44)
 Odds ratio = 85.0 with 95% CI: 22.5 to 321.8

AUC = 0.9398

7.1.2 Subset Of 178 Patients

	Probable Alzheimer's Disease	"Definite Non-Alzheimer's Disease"	Subtotals
NTP > 22	37	4	41
NTP ≤ 22	4	40	44
Subtotals	41	44	85

Agreement of Probable Alzheimer's Disease with positive NTP test = 90.2% (37/41)
 Agreement of "Definite Non-Alzheimer's Disease" with negative NTP test = 90.9% (40/44)
 Odds ratio = 92.5 with 95% CI: 21.56 to 396.80
 AUC = 0.9437

7.1.3 Subset Of 168 Patients

	Probable Alzheimer's Disease	"Definite Non-Alzheimer's Disease"	Subtotals
NTP > 22	32	4	36
NTP ≤ 22	3	39	42
Subtotals	35	43	78

Agreement of Probable Alzheimer's Disease with positive NTP test = 91.4% (32/35)
 Agreement of "Definite Non-Alzheimer's Disease" with negative NTP test = 90.7% (39/43)
 Odds ratio = 104.0 with 95% CI: 21.7 to 499.0
 AUC = 0.9535

7.2 Sponsor's Conclusions

These are below

"Thresholds of 20, 22, and 30 µg/mL provide useful prognostic assistance to the neurological workup process. For the threshold of 22 µg/mL, the percent agreement between the NTP result and the diagnosis of probable Alzheimer's Disease is 91.4% and the percentage agreement between the NTP test result and diagnosis of Definite Non-Alzheimer's Disease is 90.7%."

"A clinician who has clinically diagnosed a patient with Probable Alzheimer's Disease and receives a positive NTP test result will have added certainty that it is very likely that the diagnosis is correct. Also, a clinician who has clinically diagnosed a patient as Definite Non-Alzheimer's Disease and receives a negative NTP test result will have added certainty that it is very likely that the diagnosis is correct."

8. Additional Analyses By Agency Statistician

ch has conducted additional analyses in an effort to determine if the Urine NTP Test has any other value in the diagnosis of patients with signs and symptoms of dementia

She has used 2 approaches

8.1.1 Approach 1

The ability of the Urine NTP Test to discriminate between 2 groups from the 168-patient analysis dataset has been assessed. The 2 groups are

- Probable Alzheimer's Disease plus Possible Alzheimer's Disease
- versus
- Mild Cognitive Impairment plus "Definite Non-Alzheimer's Disease"

The following summarizes the results of her analysis

	Probable Alzheimer's Disease or Possible Alzheimer's Disease	Mild Cognitive Impairment or "Definite Non-Alzheimer's Disease"	
NTP > 22	52	22	74
NTP ≤ 22	36	58	94
	88	80	168

Agreement of Probable or Possible Alzheimer's Disease with positive NTP = 59.1% (52/88)
(95% CI: 48.1 % to 69.5%)

Agreement of Mild Cognitive Impairment or "Definite Non-Alzheimer's Disease" with negative NTP = 72.5% (58/80)
(95% CI: 61.4% to 81.9%)

She has concluded that the NTP test is better than a completely random test (i.e., a toss of the coin) in discriminating between the above 2 groups

She has also calculated pre-test/post-test probabilities and likelihood/odds ratios which are stated to show the same. Post-test probabilities for discriminating between the above 2 groupings showed a statistically significant increase compared with pre-test. Post-test odds ratios for diagnosing Probable or Possible Alzheimer's Disease, based on positive and negative test results, showed a statistically significant increase compared with pre-test

8.1.2 Approach 2

This approach is summarized by the following table and subjacent text

	Intended Use Population				
	Probable Alzheimer's Disease	Possible Alzheimer's Disease	Mild Cognitive Impairment	"Definite Non-Alzheimer's Disease"	
NTP ≤ 20	0	28	13	32	73
20 < NTP ≤ 30	18	16	15	9	58
NTP > 30	17	9	9	2	37
	35	53	37	43	168

has drawn the following conclusions from her analysis

- If the Urine NTP Test results are ≤ 20 , then the diagnosis of Probable Alzheimer's Disease may be excluded
- If the Urine NTP Test results are in the > 20 to 30 range, they may be considered inconclusive
- If the Urine NTP Test results are > 30 , then a diagnosis of "Definite Non-Alzheimer's Disease" can be excluded

9. Additional Observations

This is a limited review, which is intended to focus on the sponsor's proposed new Intended Use for the Urine NTP Test, and whether the sponsor's statistical analysis supports that Intended Use. Other aspects of this Amendment, and other submissions since the Not-Approvable letter was issued on July 22, 2004, have been or are being addressed by the primary reviewing division

Based on a limited assessment of other components of this Amendment, the following are noteworthy

- The sponsor has provided evidence to support the diagnoses of Probable Alzheimer's Disease, Possible Alzheimer's Disease, and Mild Cognitive Impairment

The evidence consists of a checklist containing the standard diagnostic criteria for each respective entity; each checklist is certified by a physician at the study site. At a teleconference with the sponsor shortly after the issuance of the Not-Approvable letter, it was indicated that such an approach would be acceptable. I have not attempted to ascertain whether the items checked in each instance are adequately supported by data contained in the Case Report Forms, laboratory and brain imaging reports, and other documents previously submitted.
- Evidence to support the assignment of individual patients to the "Definite Non-Alzheimer's Disease" category has not been provided in this submission and does not appear to have been provided since the Not-Approvable letter was issued.
- Tabulated data has been supplied for 23 patients diagnosed to have Probable or Possible Alzheimer's Disease who had not previously had brain imaging done within 12 months of providing a urine sample for NTP Test assay. A new imaging study was possible in 19 of these patients; all 19 imaging studies were done following the submission of the original Pre-Marketing Application and 16/19 after the issuance of the Not-Approvable letter. 15/19 imaging studies were done more than a year after the urine sample for the NTP test was collected.

The sponsor reports that in no instance where an imaging study was done was the diagnosis changed.

10. Comments

I will make comments under the following headings

10.1 Comments About Proposed New Intended Use And Supporting Analyses

(The comments below assume that the integrity of the study data is assured to a reasonable degree and that the assignment of study subjects to all 4 diagnostic categories is appropriate; at the present time neither assumption can be verified by this reviewer)

- The new Intended Use statement appears to imply that the Urine NTP test is intended to be used in addition to the standard panel of other diagnostic assessments, after a diagnosis of probable Alzheimer's Disease has been reached

The now-completed definitive study of this device (Protocol N600721) was not intended by design to support the above Intended Use statement.

The original Intended Use of the Urine NTP assay was as part of the early assessment of patients presenting with impaired memory or other symptoms suggesting cognitive impairment to a clinician, and was to be used prior to a definite diagnosis being made. The original Intended Use statement is as follows:

"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 a neurodegenerative disease causes of cognitive or memory disorder or dementia symptoms."

- The newly-proposed Intended Use statement implies that the test can provide additional assurance that a patient diagnosed to have Probable Alzheimer's Disease after undergoing a standard assessment, does in fact have that diagnosis. That assurance is claimed to be provided by an analysis indicating that the test can discriminate between those with probable Alzheimer's Disease and those in the "Definite Non-Alzheimer's Disease" category. However, those in the "Definite Non-Alzheimer's Disease" category in this study have, in the majority of instances, specific

diagnoses that are easily distinguished from Alzheimer's Disease, as indicated by the following table which applies to all 44 patients in this category in the entire study set (n=200). It is especially noteworthy that 24 out of 44 (54.5%) were normal individuals

Investigator Term	Number of Subjects
Normal	24
Age-related memory impairment	3
Depression	2
Severe depression, possible schizophrenia	1
Amnesic disorder (not amounting to dementia) secondary to cerebrovascular disease	1
Old stroke, possible depression, possible post-traumatic stress disorder	1
Hypothyroidism	1
Prior head injury, multiple medications, obstructive sleep apnea, metabolic encephalopathy	1
Diabetes mellitus, decreased hearing	1
Anxiety disorder	1
Cyclothymia	1
Bipolar disorder, depression	1
Diabetic peripheral neuropathy*	1
Epilepsy	1
Depression, anxiety	1
Anxiety, possible age-related memory impairment	1
Bipolar	1
Pseudodementia due to depression	1

*No complaint related to memory/cognition, according to Case Report Form

- When a diagnosis of Probable Alzheimer's Disease is made in clinical practice using the standard current approach, conditions that remain to be excluded are a number of entities such as Frontotemporal Dementia, Dementia With Lewy Bodies, and pseudodementia due to depression; these conditions may be difficult to exclude using the customary approach for making a diagnosis of Probable Alzheimer's Disease. These entities are minimally represented or not represented at all in the "Definite Non-Alzheimer's Disease" category (several of these entities do require autopsy diagnosis for confirmation, although clinical criteria exist). A diagnosis of Probable Alzheimer's Disease, by definition, implies that other conditions such as the clinically normal state, most psychiatric conditions, "age-related memory impairment," hypothyroidism and other entities have been excluded, as have Possible Alzheimer's Disease and Mild Cognitive Impairment. [In clinical practice, it is also seldom difficult to distinguish the vast majority of entities in the table above from Alzheimer's Disease]

Thus, I cannot agree with the sponsor's contention that a Urine NTP Test result > 22 µg/mL in a patient diagnosed to have Probable Alzheimer's Disease adds certainty to the diagnosis. In fact, the only means of establishing that any Urine NTP Test result adds certainty to the diagnosis is by enrolling patients with Probable Alzheimer's Disease in a study, measuring their Urine NTP level and then attempting to confirm the diagnosis further by other means, keeping in mind that definitive

confirmation requires autopsy. [Note that, ultimately, the diagnostic process may be further confounded by the frequent co-existence of the neuropathological changes of Alzheimer's Disease in patients who have Dementia With Lewy Bodies.]

10.2 Comments About Additional Analyses

(The comments below assume that the integrity of the study data is assured to a reasonable degree and that the assignment of study subjects to all 4 diagnostic categories is appropriate; at the present time neither assumption can be verified)

- There would seem to be little clinical utility in discriminating between the (Probable Alzheimer's Disease plus Possible Alzheimer's Disease) and (Mild Cognitive Impairment plus "Definite Non-Alzheimer's Disease") populations
- Based on analysis, it does appear that a urine NTP result $< 20 \mu\text{g/mL}$ excludes a diagnosis of Probable Alzheimer's Disease, at least in the population enrolled in this study. However, the practical utility of this observation may not be applicable to the scenario in which the sponsor has envisaged using the test, i.e., in patients in whom a diagnosis of probable Alzheimer's Disease has already been made using the standard diagnostic approach. The utility of the test in distinguishing patients with Alzheimer's Disease from those with other degenerative dementias such as frontotemporal dementia should, for example, have been established; that is currently not the case
- The clinical utility of excluding a diagnosis of "Definite Non-Alzheimer's Disease" based on a test result $> 30 \mu\text{g/mL}$ is questionable, under the conditions under which the use of this test is now envisaged. The "Definite Non-Alzheimer's Disease" category in this study does not include patients with conditions that Probable Alzheimer's Disease needs to be distinguished from in the clinical setting.

10.3 Additional Comments

As this review was mainly confined to addressing a limited segment of this Amendment, I have not been able to determine the full extent to which the major concerns (listed below) that I had earlier about the data in this submission have been addressed

- The process by which diagnoses were made (i.e. the process by which patients were assigned to the 4 diagnostic categories: Probable Alzheimer's Disease, Possible Alzheimer's Disease, Mild Cognitive Impairment, and "Definite Non-Alzheimer's Disease").

The primary reviewing division (Division of Immunology and Molecular Diagnostics) has, however, been reviewing all data submitted by the sponsor since the issuance of the Not-Approvable letter of July 22, 2004, and may be able to address these concerns to a greater extent than I can

I do, however, note that there is still very inadequate information available as to the mechanism for assigning patients to the "Definite Non-Alzheimer's Disease" category

11. Main Conclusion

The Intended Use of the Urine NTP Test as proposed in the current submission is not supported by either the design and conduct of Study N600721 or the new analysis included in this Amendment

rbm 2/6/05

cc:

HFD-120

PMA 040010

TAB 7

FDA Statistical Review

Date: June 19, 2005

Subject: Statistical comments on the Amendment to PMA, P040010, Urine Neural Thread Protein (NTP) test by Nymox (obtained on May 10,2005).

Background

1. In the original PMA submitted in March of 2004, the sponsor provided the following intended use/indication 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.”

2. In the amendment submitted by the sponsor in response to the issues raised in the not-approvable letter sent by the FDA to the sponsor in July 2004, the sponsor proposed the intended use/indication for use of their test in the following form:

“The intended use for the Urine NTP Test is to provide diagnostic information to be used in the assessment of patients with signs and symptoms of dementia. Test results, when considered in conjunction with other tests will add more certainty for the confirmation of a probable Alzheimer’s disease cause of cognitive or memory disorder or dementia symptoms. The urine NTP test is not a stand-alone diagnostic and should not be interpreted without history, physical and neurological examination, and all available medical and laboratory data.”

3. In the amendment submitted by the sponsor in April and May 2005, the sponsor changed the previous intended use/indication for use and proposed the intended use/indication for use:

“Intended-Use: The urine NTP Kit is designed to measure levels of neural thread protein in urine specimens from patients presenting with cognitive complaints or other signs and symptoms of suspected Alzheimer’s disease (AD). Results from Urine NTP are intended for use, in conjunction with and not in lieu of current

standard diagnostic procedures, to aid the physician in the diagnosis of Definite Non-AD versus Probable AD, Possible AD, or MCI.

Indication for Use: Urine NTP measurement can be used as part of diagnostic risk assessment for presence or absence of Definite Non-AD. Urine NTP has 91% specificity and is indicated for use as an adjunctive aid to help clinicians rule out definite non-AD as part of the overall diagnostic categorization; that is, only 9% of the cases of definite non-AD have elevated urine NTP levels. Urine NTP has 60% sensitivity for the diagnoses of probable AD, Possible AD, and MCI (as per NINCDS-ADRDA criteria, and criteria of the Quality Standards Subcommittee of the American Academy of Neurology); hence an elevated urine NTP level may help the clinician's decision for the need of further diagnostic workup (such as specialist consultations, imaging, in-depth neuropsychological testing, EEG and other testing procedures) to gain further diagnostic clarity".

Brief description of study

Data collected from this study were the NTP test results along with an assigned diagnostic category:

- (1) Probable Alzheimer's disease (Prob AD), using NINCDS-DRDA criteria for probable AD;
- (2) Possible Alzheimer's disease (Poss AD), using NINCDS-ADRDA criteria for possible AD;
- (3) Mild cognitive impairment (MCI), using criteria of the Quality Standards Subcommittee of the American Academy of Neurology; and
- (4) Definite non-Alzheimer's disease (Def Non-AD).

Also, the sponsor provided the NTP results for 122 apparently healthy subjects.

Subjects Accountability:

The total number of subjects with signed informed consent was 366. After informed consent was received, a first morning urine sample was obtained from each subject. The NTP test results are invalid for urine sample under any of the following circumstances (if urine was provided): 1) non-first morning sample, 2) frozen sample, 3) urinary tract infection or contaminated urine, 4) glycosuria, 5) proteinuria, 6) presence of nitrites, 7) urine creatine <50 mg/dL or >225 mg/dL. Urine samples with invalid results were excluded and repeat urine samples were requested. Subjects who were unable to provide an acceptable first morning urine sample were excluded.

The 45% of the subjects (166 out of 366) were excluded from the study by the following reasons:

- 36.3% (133 out of 366) – no acceptable urine sample provided;
- 4.9% (18 out of 366) – urine sample was provided but no clinical evaluation;
- 4.1% (15 out of 366) – urine sample was provided before or after study.

So, more than **36%** (133/366) of the subjects from the intended use population were unable to obtain the valid NTP test results because no acceptable urine samples were provided.

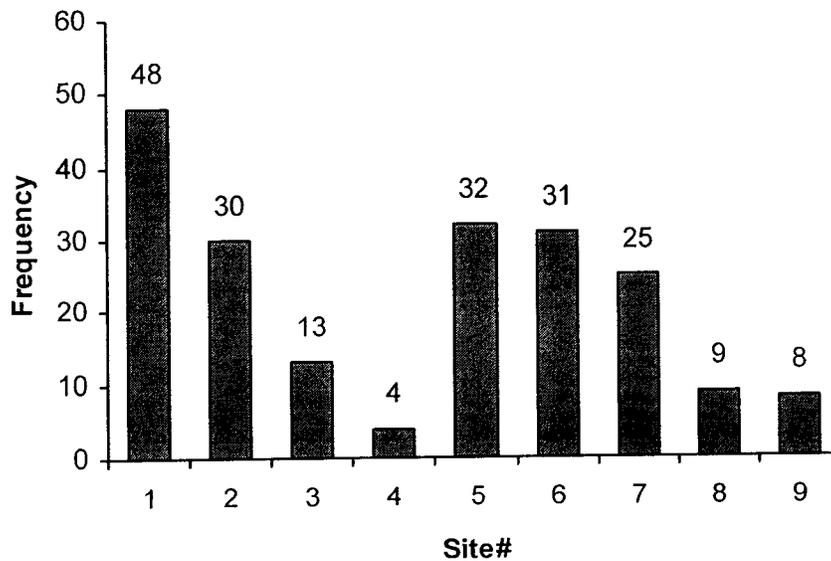
Cutoff:

The sponsor checked the cutoff values from 20 µg/mL to 30 µg/mL and found that the cutoff of 21.7 µg/mL is optimal for the data of this clinical study. The cutoff of 22 µg/mL was proposed for labeling. So, the negative NTP values were defined as NTP values ≤ 22 µg/mL and positive NTP values were defined as NTP values >22 µg/mL.

Objective:

The objective of the study was to estimate the percent of agreements of the NTP values with the clinical diagnoses.

The sponsor’s clinical study involved 9 centers. The data submitted by the sponsor included 200 patients. The distribution of numbers of the patients across the different sites was following: (48, 32, 31, 30, 25, 13, 9, 8, and 4) with the largest numbers of patients at site #1 in Coatesville, PA (48 patients) and at site #5 in Miami, FL (32 patients).



The data submitted by the sponsor for the original PMA (200 patients) is presented by the following table:

Table 1: All 9 Sites

	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

Sponsor's Statistical Analysis

1. The basic points of the sponsor's statistical analysis in the Amendment 005 submitted in April 2005 are (citations from 3-5 of the amendment):
 - "The study population provides unbiased estimates of the prevalences for the intended use population:
 - 78% of the patients were Prob AD, Poss AD, or MCI;
 - 22% of the patients were Def Non-AD".
 - "The PPV for the NTP measurements (96%) is extremely high, and represents a 23% gain in accuracy ($[(96-78)/78] 100\%$) by having the NTP measurement".
 - "The NPV (39%) shows that, given a normal NTP level, there is 77% gain in accuracy by having the NTP measurement for Def Non-AD ($[(39-22)/22] 100\%$)".
 - "Information Benefit: the existing data clearly answers the question whether urine NTP levels add information for its intended use (information "with NTP levels" vs. information "without NTP levels"). "With NTP levels" (PPV=96% and NPV=39%) the information to the clinician clearly is better than "without NTP levels" (prior probability of 78% and 22% respectively).

The sponsor also considered diagnosis of Prob AD versus Poss AD, MCI, or Def Non-AD "... (sensitivity=89%, specificity=67%). The study demonstrates a very high estimate of NPV (94%)." (Citation from page 4).

2. In the Amendment submitted in May of 2005, the sponsor provided a bootstrapping of the data set of 200 patients. By using the bootstrap technique, the sponsor provided mean and median NTP values for each diagnostic category. The parameters of sensitivity, specificity, PPV and NPV were calculated for Prob AD, Poss AD, or MCI vs. def Non-AD and for Prob AD vs. Poss AD, MCI, or Def Non-AD. Using the bias corrected and accelerated method, the sponsor calculated 95% one-sided confidence intervals for these parameters (see table on page 10 of the amendment).

Comments

1. Use of the terms "Sensitivity" and "Specificity" in this study

Sensitivity and specificity are useful performance characteristics of the test for detection of a target condition "Diseased" if

- There are only two groups in the intended use population, Diseased and Non-Diseased;
- The categorization of a case as Diseased/Non-Diseased can be made by the "reference standard", a method or combination of methods that the clinical community relies upon for diagnosis and which is regarded as having negligible risk of either false positive or false negative result. The reference standard should not give intermediate or equivocal results.

In this study, the NTP test is compared to the four diagnostic categories not to the pathological diagnosis. It is well known that these diagnostic categories when compared to the pathological diagnosis do not have "negligible FP or FN results".

Indeed, for example, the paper by D. Blacker et al.¹ provided the following table of performance (table 3 on page 71 of the paper):

Diagnostic category	Pathological Diagnosis*		Total
	AD present	AD absent	
Prob AD	51	6	57
Poss AD	14	5	19
Non-AD	15	29	44
Total	80	40	120

*Pathological diagnoses were based on the practice of each institution at the time the autopsy was performed.

The sensitivity of diagnostic category “Prob AD” was 64% (51/80) and false positive rate was 15% (6/40). For diagnostic categories Prob AD or Poss AD, the sensitivity was 81% (65/80) and false positive rate was 28% (11/40). So, the diagnostic categories in the clinical study cannot be considered as the reference standard for detection AD and therefore the use of the terms “sensitivity and specificity for detection of AD” can be misleading. The use of terms as Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of NTP test results with corresponding diagnostic categories is appropriate for this clinical study.

2. Reporting the Performance of NTP test only for Two Categories: Prob AD and Def Non-AD

Reporting of the performance of NTP test only for two categories as, for example, PPA of NTP with Prob AD is 89% (51/57) and NPA of NTP test with Def Non-AD was 91% (40/44), can be misleading. One can think these two estimates can provide some information about performance of NTP test with regard to reference standard, but these estimates can be very biased for the following reasons:

- 1) Not all Prob AD subjects are the subjects with AD positive pathological diagnosis and not all Def Non-AD subjects are the subjects with AD negative pathological diagnosis (see comment above) and
- 2) Prob AD subjects does not present a *random sample* of the subjects with AD positive pathological diagnosis. So, one cannot be sure that the diagnostic category of Prob AD subjects is a representative sample of AD pathologically positive cases and includes cases that were complex or difficult to diagnose. Similarly, for category Def Non-AD. When a study sample of Diseased subjects is not a representative sample of all Diseased cases, the *spectrum bias* can occur. Non-random selection of the Diseased subjects can enhance the apparent accuracy of the test.

3. Intended Use Population

¹ Blacker D, Albert MS, Bassett SS, Go RC, Harrel LE, Folstein MF. Reliability and validity of NINCDS criteria for Alzheimer's disease. *Arch.Neurology.* 1994; 51:1198-1204.

In the study, more than **36%** (133/366) of the subjects from the intended use population were unable to obtain a valid NTP test results because no acceptable urine samples were provided. Therefore, the intended use population, “patients presenting with cognitive complaints or other signs and symptoms of suspected Alzheimer’s disease (AD)”, is limited to the patients able to provide a urine sample for a valid NTP results.

4. NTP Distinction Between (Prob AD, Poss AD, or MCI) vs Def Non-AD
NTP value > 22 µg/mL: “Rule-Out” Def Non-AD

The intended use population was divided in the two groups: subjects with diagnosis of either Prob AD or Poss AD or MCI and subjects with diagnosis of Def Non-AD. The performance of NTP test in the study with regard to these two groups is presented by the following table for the data set of 200 subjects (data from all 9 sites

	Data Set of 200 Patients
Positive Percent Agreement (PPA)	60.3% (94/156) 95% two-sided CI: (52.4%; 67.6%)
Negative Percent Agreement (NPA)	90.9% (40/44) 95% two-sided CI: (78.8%; 96.4%)
PPV-like	95.9% (94/98) 95% two-sided CI: (90.0%; 98.4%)
NPV-like	39.2% (40/102) 95% two-sided CI: (30.3%; 48.9%)
	NTP Test is “Informative” (better than a random test) PPA + NPA = 151.2% with 95% CI: (137.4%; 161.5%) AUC = 0.760 with 95% CI: (0.691; 0.830)

For the NTP > 22 µg/mL, the estimate of PPV was 95.9% with 95% CI: 90.0% to 98.4% for 200 subjects.

In other words, *if the diagnosis of Def Non-AD is ruled-out on the basis of positive NTP result ($>22 \mu\text{g/mL}$), then the probability that an incorrect decision was made can be up to 10%.*

For the subjects with negative NTP results, the diagnosis of Def Non-AD is correct only 39% of the time (according to the 95% CI, this probability can be low as 30.3%).

**5. NTP Distinction Between Prob AD vs. (Poss AD, MCI, or Def Non-AD)
NTP value $\leq 22 \mu\text{g/mL}$: “Rule-Out” Prob AD**

The intended use population was divided in the two groups: subjects with diagnosis of Prob AD and subjects with diagnoses of either Poss AD or MCI or Def Non-AD. The performance of NTP test in the study with regard to these two groups is presented by the following table for the data set of 200 subjects (data from all 9 sites)

	Data Set of 200 Patients
Positive Percent Agreement (PPA)	89.5% (51/57) 95% two-sided CI: (78.9%; 95.1%)
Negative Percent Agreement (NPA)	67.1% (96/143) 95% two-sided CI: (59.1%; 74.3%)
PPV-like	52.0% (51/98) 95% two-sided CI: (42.3%; 61.7%)
NPV-like	94.1% (96/102) 95% two-sided CI: (87.8%; 97.3%)
	NTP Test is “Informative” (better than a random test) PPA + NPA = 156.6% with 95% CI: (143.6%; 166.5%) AUC = 0.818 with 95% CI: (0.761; 0.875)

For the NTP $\leq 22 \mu\text{g/mL}$, the estimate of NPV was 94.1% with 95% CI: 87.8% to 97.3% for 200 subjects.

In other words, *if the diagnosis of Prob AD is ruled-out on the basis of the negative NTP results ($\leq 22 \mu\text{g/mL}$), then the probability that the incorrect decision was made may be up to 12.2%.*

For the subjects with positive NTP results, the diagnosis of Prob AD is correct only 52% of the time (according to the 95% CI, it can be low as 42.3%).

6. Dependence of PPV and NPV on Prevalence

The both predictive values depend not only on the performance of the test in the two groups but also on the prevalence of these groups in the intended use population.

6.1 For the prevalence of group (Prob AD, Poss AD, or MCI) of 78%, the PPV for the positive NTP result was 95.9%. The prevalence of 78% presented the prevalence averaged over all 9 sites.

In the study, the prevalence of the group (Prob AD, Poss AD, or MCI) showed the following variability:

Site	Total	Prevalence of (Prob AD, Poss AD, or MCI)
1	48	48% (23/48)
2	30	83% (25/30)
5	32	97% (31/32)
6	31	81% (25/31)
7	25	84% (21/25)
Other	34	91% (31/34)
Total	200	78% (156/200)

The range of the prevalence of the group (Prob AD, Poss AD, or MCI) for the different clinical sites was from 48% to 97%. For the prevalence of 48% (under assumption that the estimates of PPA and NPA are valid for this prevalence), the estimate of PPV will drop to 86% compared with the estimate of PPV of 96% for the average prevalence of 78%.

6.2 For a prevalence of Prob AD of 29%, the NPV for the negative NTP result was 94.1%. The prevalence of 29% presented the prevalence averaged over all 9 sites. In the study, the prevalence of the Prob AD showed the following variability:

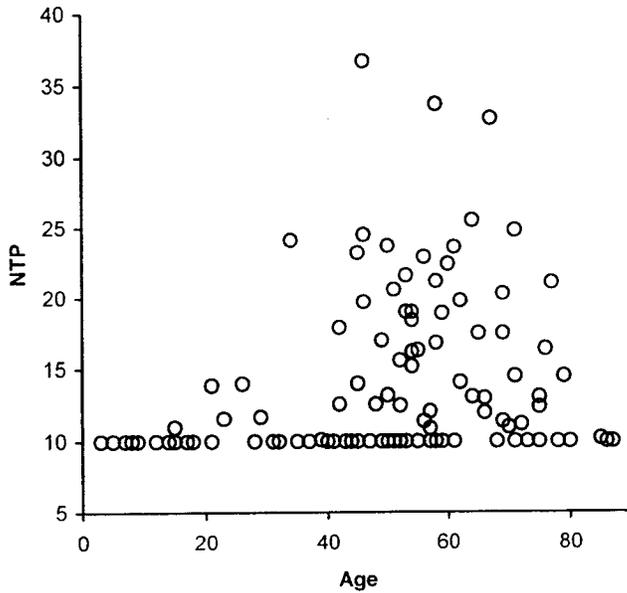
Site	Total	Prevalence of Prob AD
1	48	10% (5/48)
2	30	37% (11/30)
5	32	69% (22/32)
6	31	26% (8/31)
7	25	23% (7/31)
Other	34	12% (4/34)

Total	200	29% (57/200)
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The range of the prevalence of the Prob AD for the different clinical sites was from 10% to 69%. For the prevalence of 69% (under assumptions that the estimate of PPA and NPA are valid for this prevalence), the estimate of NPV will drop to 74% compared with the estimate of NPV of 94% for the average prevalence of 29%.

7. Reference Interval

122 subjects with no history of cognitive complains or symptoms were assayed. The scatterplot of NTP values by subject's age is shown below.



There were statistically significant differences in the distributions of the NTP values for the subjects of age 3-39 years old and for subjects 40-87 years old.

	Age 3-39 (29 subjects)	Age 40 – 87 (92 subjects)
NTP value <10 µg/mL	75.9% (22/29)	43.5% (40/92)
NTP value > 20 µg/mL	3.4% (1/29)	17.4% (16/92)
NTP value > 22 µg/mL	3.4% (1/29)	12.0% (11/92)
NTP value > 30 µg/mL	0% (0/29)	3.3% (3/92)

For the age group matched to the intended use population, 12% of the subjects had NTP values above 22 µg/mL.

TAB 8

Draft Questions for Panel and Voting Options

Draft Questions for Panel:

1. Has the NTP test been studied in an appropriate population and with appropriate diagnostic criteria to allow for determination of clinical performance in the proposed intended use setting?
2. Can the NTP test be used to guide selection of steps for evaluation of patients with Alzheimer's disease in the differential diagnosis?
3. Does the NTP test add certainty to the diagnosis or exclusion of Alzheimer's disease in the intended use population?
4. Is the NTP test a safe and effective tool for the diagnosis of patients with Alzheimer's disease or other forms of dementia?

Panel Recommendation Options

For

Premarket Approval Applications

The Medical Device Amendments to the Federal Food, Drug and Cosmetic Act (Act), as amended by the Safe Medical Devices Act of 1990, allows the Food and Drug Administration to obtain a recommendation from an expert advisory panel on designated medical device premarket approval applications (PMAs) that are filed with the Agency. The PMA must stand on its own merits and your recommendation must be supported by safety and effectiveness data in the application or by applicable publicly available information. Safety is defined in the Act as reasonable assurance, based on valid scientific evidence that the probable benefits to health {under conditions on intended use} outweigh any probable risks. Effectiveness is defined as reasonable assurance that, in a significant portion of the population, the use of the device for its intended uses and conditions of use {when labeled} will provide clinically significant results.

Your recommendation options for the vote are as follows:

1. **APPROVAL** – If there are no conditions attached.
2. **APPROVABLE with conditions** – The panel may recommend that the PMA be found approvable subject to specified conditions, such as physician or patient education, labeling changes, or a further analysis of existing data. Prior to voting, all of the conditions should be discussed by the Panel.
3. **NOT APPROVABLE** – The panel may recommend that the PMA is not approvable if:

- the data DO NOT provide a reasonable assurance that the device is safe,

OR

- the data DO NOT provide a reasonable assurance that the device is effective, under the conditions of use prescribed, recommended, or suggested in the proposed labeling.

Following the voting, the Chair will ask each panel member to present a brief statement outlining the reasons for their vote.

TAB 9

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TAB 10

Sponsor's Briefing Document



PMA P040010

Urine NTP Kit

BRIEFING DOCUMENT

REDACTED FOR WEB POSTING

Prepared by

Nymox Pharmaceutical Corporation

for the

FDA Immunology Devices Advisory Panel Meeting

July 15, 2005

TABLE OF CONTENTS

I. Executive Summary.....2

 1. Medical Need2

 2. The Device2

 3. Safety of the Device3

 4. Effectiveness of the Device3

 5. Clinical Study3

 6. Risk/Benefit Analysis.....4

 7. Proposed Labeling: Intended Use and Indications For Use5

 8. Limitations.....5

 9. Conclusion.....5

II. Clinical Study6

 1. Investigators and Centers6

 2. Summary of Clinical Study8

 A. Design8

 B. Clinical Study Results.....10

 C. Summary of Biostatistical Analysis of Results12

IV. Summary of Non-Clinical Studies.....26

V. Scientific Background of NTP Biomarker28

VI. References29

VII. Speakers Representing Sponsor at Advisory Panel Meeting33

VIII. Proposed Labeling.....34

I. Executive Summary

Alzheimer's Disease (AD) is the most common form of dementia in the elderly. It is the fifth leading cause of death in the age range of 65 or older, with a prevalence estimated at 4.5 million in the U.S.

1. Medical Need

Diagnostic evaluation of patients with suspected AD is imperfect. While experts and tertiary care centers report high accuracy (>80% sensitivity and specificity), the vast majority of cases are seen in the community, where these figures are drastically lower. According to many published studies (1-7), up to 75% or more of AD cases are initially missed by family physicians and non-specialists.

Earlier diagnosis translates into earlier and better care. If the diagnosis is not AD, earlier clarification may lead to effective treatments or counselling for conditions such as depression or metabolic disease. If the diagnosis is AD, there are some available drug treatments, long term planning, nursing care, counselling, family care, and other options which collectively have a helpful impact on the patient and their family. With earlier diagnosis, planning and care can be optimized earlier and institutionalization may be delayed, allowing for improved quality of life for a significant time, both for patient and family.

Thus there is a significant need for technologies that can improve the currently imperfect situation. A safe and non-invasive modality which adds useful information about suspected AD and which can be used without special training by the general physician will prove to be extremely valuable.

2. The Device

The urine NTP device is an indirect ELISA format assay which measures NTP in a first morning urine sample. Coated plates bind NTP in urine in competition with labeled rabbit anti-mouse IgG. The normal reference range is ≤ 22 $\mu\text{g/mL}$.

Background Basic Science

The predicted 375 amino acid sequence for NTP was derived from cDNA extracted from postmortem AD brain (8). NTP is believed to be a membrane-spanning Alu-containing phosphoprotein. Monoclonal specific anti-NTP antibodies raised against cloned recombinant NTP selectively stain degenerating neurons in AD brain, in relation to neuritic plaques and neurofibrillary tangles (9-11). NTP *in vitro* leads to neuronal cell sprouting and premature cell death (8,12). In transgenic animal studies, NTP leads to behavioral abnormalities and reproduces many of the histological changes found in AD brain (cell death, amyloid formation, phospho-tau production). In the past 15 years, there have been 27 peer-reviewed published studies about NTP and the above relevance to AD (8-32, 66, 77). NTP has pathophysiological significance and is clearly associated with the underlying disease processes (structural hallmarks of plaques and tangles) in AD brain.

Regulatory History of the Device

The urine NTP assay has been available in the U.S. since 2000 as a reference laboratory test through the Sponsor's CLIA-certified clinical reference laboratory. It has been used safely on over 1500 patients, with data published in the peer-reviewed literature showing >85% agreement with clinical diagnosis, including cases with one year clinical follow-up. This PMA is for approval of a kit version of the NTP assay to allow other laboratories to perform the assay. The kit version of the NTP assay received the CE Mark from the EU in 2004.

Anticipated Use

The device is anticipated to have utility as an early aid to diagnosis, to be used as an indicator for the non-specialist physician to help in the decision to proceed to further diagnostic evaluation, including referral and more testing. The device is **not** envisaged to be a stand-alone diagnostic, and it is **not** envisaged to provide advanced complex information (such as diagnosis of mixed, incompletely understood, or controversial neurological entities).

3. Safety of the Device

The device is a kit version of a non-invasive *in vitro* urine assay that poses no safety concerns for patients. The assay has been performed on over 1500 patient samples since 2000 without incident.

4. Effectiveness of the Device

In our blinded prospective multi-center U.S. trial, 96% of the instances of elevated urine NTP levels were found in patients with probable AD, possible AD, and Mild Cognitive Impairment (MCI). Elevated NTP levels (> 22 µg/mL) had a percentage agreement of 89% with the clinical diagnosis of probable AD. Urine NTP had 91% specificity (9% of cases of definite non-AD had elevated urine NTP levels).

5. Clinical Study

Clinical Study Design

To determine utility, in accordance with FDA guidance, the clinical study was designed to test the device in the context in which it is anticipated to be used (early stage evaluation of patients suspected of having AD). Therefore, consecutive unselected patients who were undergoing diagnostic evaluation were first assayed for their urine NTP level, which was subsequently compared with the evaluation by experienced specialists with full facilities using current standard of care practice, according to well defined homogeneous standard criteria. The study was designed to answer the following question: In the typical anticipated clinical context, based on comparison with diagnoses from experienced specialists using standard criteria, do urinary NTP levels add useful information? In other words, is the information "with NTP levels" significantly better than the information "without NTP levels"? Therefore, if "with NTP levels" is shown to add useful information, then the non-specialist (without extra training) will have access to a modality that will be helpful for all concerned.

Clinical Study Results

Two hundred subjects fulfilled inclusion criteria and completed the study. The table below summarizes the study results.

Table E1: Summary of Study Results

Cutoff = 22 µg/mL	Clinical Diagnosis Category				Totals
	Probable AD	Possible AD	MCI	Definite Non-AD	
NTP > 22	51	21	22	4	98
NTP ≤ 22	6	35	21	40	102
Totals	57	56	43	44	200

Biostatistics

The clinical study results clearly demonstrate the ability of NTP measurement to discriminate between probable AD and definite non-AD using the device’s cut-off of 22 µg/mL. At this cut-off value, the sensitivity (% agreement) of NTP measurement for probable AD is 89.5% and the specificity (% agreement) of NTP measurement for definite non-AD is 91%. The positive predictive value (PPV) for an elevated NTP measurement to indicate probable AD or possible AD or MCI (i.e. not definite non-AD) is 96%. Consistent with clinical study design expectations, elevated NTP levels had intermediate percentage agreement with the diagnosis of Mild Cognitive Impairment (MCI) (51%) and Possible AD (37.5%).

The difference in the mean NTP measurements among the diagnostic groups was statistically significant (p <0.0001; Kruskal-Wallis test). Mean NTP result decreased with decreasing likelihood of AD. There was no effect of age on NTP level within each diagnostic group (R² < .036) or overall (R² = .025). The distribution of the NTP assay results by gender over all 4 groups showed no significant differences between the genders (p=0.75; Wilcoxon test).

6. Benefit/Risk Analysis

The measurement of urine NTP levels has a highly favorable benefit-risk profile as part of the diagnostic workup of patients with suspected AD. Urine NTP level has 91% specificity and 96% positive predictive value as a measurement helping the clinician in the decision to pursue or not to pursue referrals and other diagnostic procedures. As an *in vitro* urine assay, the device poses no risk, and certainly has considerably less risk or radiation or invasiveness than currently used diagnostic procedures such as spinal fluid assays or radiological procedures.

7. Proposed Labeling: Intended Use and Indications For Use

“Intended-Use: The Urine NTP Kit is designed to measure levels of neural thread protein in urine specimens from patients presenting with cognitive complaints or other signs and symptoms of suspected Alzheimer’s disease (AD). Results from the Urine NTP Kit are intended for use, in conjunction with and not in lieu of current standard diagnostic procedures, to aid the physician in the diagnosis of Definite Non-AD versus Probable AD, Possible AD, or MCI.

Indications for Use: Urine NTP measurement can be used as part of diagnostic risk assessment for presence or absence of Definite Non-AD. Urine NTP has 91% specificity and is indicated for use as an adjunctive aid to help clinicians rule out definite non-AD as part of the overall diagnostic categorization; that is, only 9% of cases of definite non-AD have elevated urine NTP levels. Urine NTP has 60% sensitivity for the diagnoses of probable AD, possible AD, and MCI (as per NINCDS-ADRDA criteria, and criteria of the Quality Standards Subcommittee of the American Academy of Neurology); hence an elevated urine NTP level may help the clinician’s decision for the need of further diagnostic workup (such as specialist consultations, imaging, in depth neuropsychological testing, EEG and other testing procedures) to gain further diagnostic clarity.”

8. Limitations

Deriving useful information about a neurodegenerative disease of the brain from a simple, painless, risk-free urine sample is remarkable. However, a single urine test cannot reasonably be expected to answer unanswerable questions, such as diagnosis of rare diseases, mixed pathology disease states, or other incompletely understood, complex or controversial entities.

9. Conclusion

Measurement of urine NTP levels provides useful information. The positive predictive value of an elevated NTP level is 96%. The device, which is a kit version of an *in vitro* urine assay used with over 1500 patients to date, poses no safety issues. In comparison, it is considerably safer than various imaging procedures which involve radiation, and invasive spinal fluid assays, all of which are currently used in AD diagnosis. “With NTP” provides an increment of 23% in positive predictive value and an increment of 78% in negative predictive value, in comparison to “without NTP” prior probability. A urine assay which provides useful information about a neurodegenerative CNS disease is remarkable.

The urine NTP measurement provides useful information that can help the non-specialist physician in the decision to pursue or not to pursue referrals and further testing for cases of suspected AD. This represents a highly significant unmet practice need.

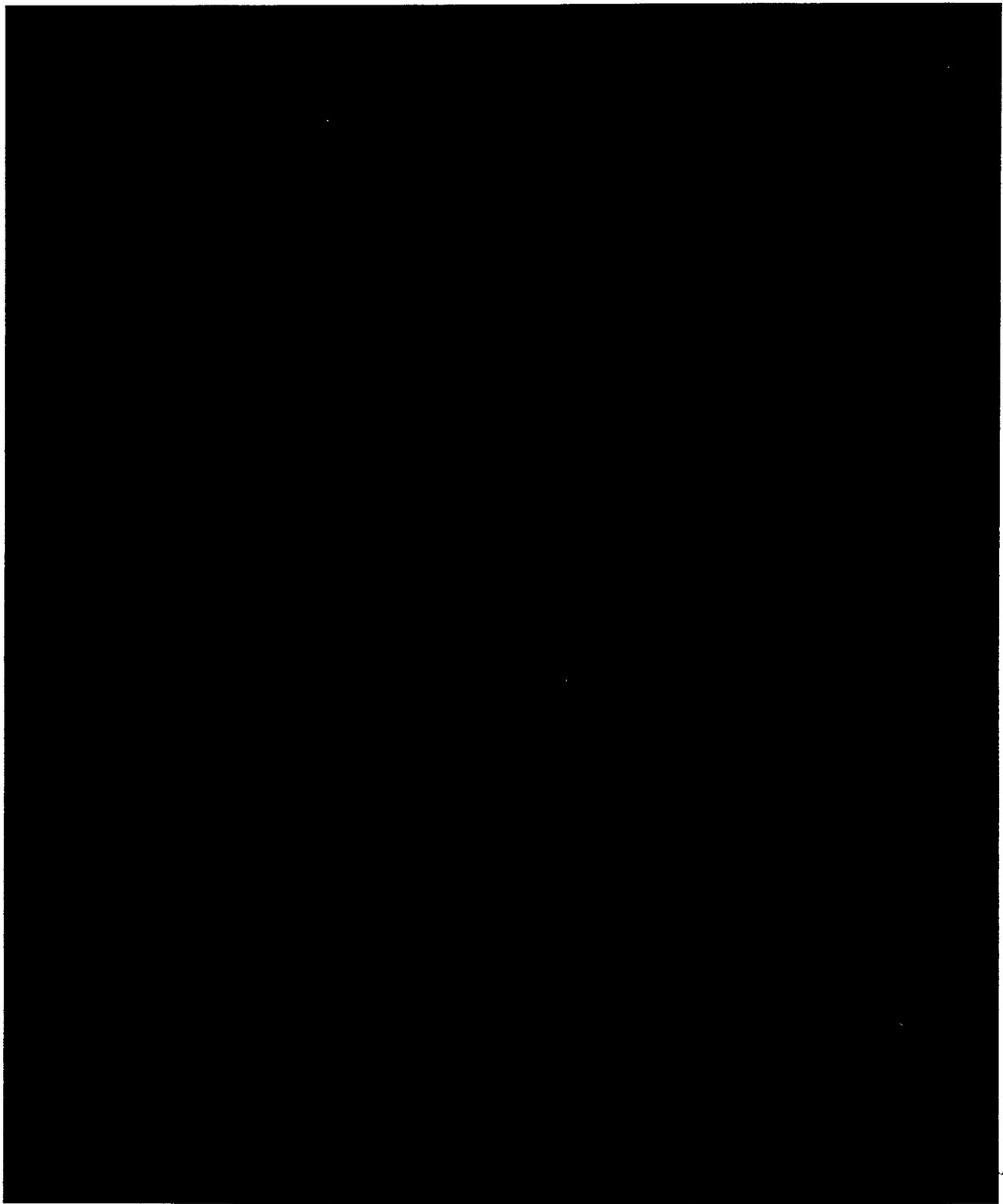
II. Clinical Study

1. Investigators and Centers

All investigators in the clinical study are highly experienced specialists with long and extensive expertise in the diagnosis of AD. Investigators at each site had affiliations to academic institutions, and 8 out of 9 are currently clinical staff at teaching hospitals and/or medical schools in the US. Seven out of nine are Chairmen of the Department or Medical Directors in their institutions. The clinical diagnoses from these highly competent and experienced specialists are representative of the top level of current U.S. specialist standard of care for the clinical diagnosis of AD.

The 9 study centers are located in 8 different states (New York, Pennsylvania, Florida, Louisiana, Ohio, Minnesota, Oklahoma, Arizona). Each study center has full access to all necessary facilities (such as imaging facilities, laboratory facilities, neuropsychology, EEG, etc.).





2. Summary of Clinical Study

A. Design

The clinical study was designed as a blinded trial of community patients undergoing evaluation by specialist clinics.

The clinical study was designed as a 9-site prospective blinded trial with an unselected, “all comers” heterogeneous population of patients who had been referred to specialist clinics for evaluation of suspected AD. Institutional review board approvals for the study were obtained for each participating study center. All subjects gave written informed consent to provide a urine sample for NTP testing and to participate in the trial. Subjects provided blind-coded urine samples for NTP testing and then underwent clinical evaluation by a specialist team blinded to the results of the NTP testing. At the completion of the trial, the clinical diagnoses and NTP measurement results were unblinded and compared.

This design was chosen after discussions with FDA in order to test the device in the realistic clinical context in which it was intended to be used.

Inclusionary criteria required subjects to be referred to or presenting to specialist dementia/memory disorder/ Alzheimer's disease clinics or units for assessment of cognitive or memory disorder symptoms or complaints or concerns, or dementia, to rule out or rule in Alzheimer's disease. Subjects were not recruited specifically for the study (i.e., no subject was called in specifically to be tested), but rather were tested in the normal course of their clinical workup. All subjects gave their written informed consent. Each subject was required to undergo comprehensive clinical assessments as outlined in the informed consent and set out in the clinical trial protocol, and was to be determined to have probable AD, possible AD, mild cognitive impairment (MCI) or definite non-AD in accordance with conventional criteria set out in the clinical trial protocol and case report form.

After informed consent was received, a first morning urinary void sample was obtained from each subject. Urine samples were blind coded on site and sent to the central core laboratory for NTP measurement. All laboratory procedures were done without any knowledge of subjects' identities, or diagnoses. Urine samples which were determined at the central core laboratory to be abnormal (contaminated, proteinuric, or hematuric), or to have creatinine concentration <50 mg/dL (indicating not a first morning void) or >225mg/dL, were excluded and a repeat urine sample was requested.

Subjects who were unable, for whatever cause, to provide an acceptable first morning urine sample were excluded.

The study had two clearly defined data points.

The clinical study had only 2 sets of data points (2 variables): 1) urinary NTP level, measured in a validated and inspected central core laboratory, and 2) clinical diagnosis from qualified, experienced, reliable specialist clinics.

The gold standard for clinical diagnosis was the NINCDS-ADRDA criteria for probable AD. The NINCDS-ADRDA criteria for probable AD are validated and conventional standards for clinical diagnosis widely accepted for use both in the clinical setting and in AD drug trials.

Clinical diagnoses were categorized as follows:

1. Probable AD (using NINCDS-ADRDA criteria for probable AD);
2. Possible AD (using NINCDS-ADRDA criteria for possible AD);
3. Mild cognitive impairment (MCI) (using criteria of the Quality Standards Subcommittee of the American Academy of Neurology);
4. Definite Non-AD.

Both the possible AD and the MCI categories would include an indeterminate percentage of AD cases. Possible AD also includes cases where the diagnosis was mixed (e.g. AD plus Parkinson's disease or AD plus vascular dementia).

The definite non-AD category comprised cases where AD could definitely be ruled out. The clinical trial protocol anticipated that the definite non-AD category would include diagnoses such as depression, anxiety, normal aging, drug side effect, metabolic disorder, infectious disease, hormonal abnormality, epilepsy, schizophrenia, alcoholism, tumor, subdural, multiple sclerosis, etc.

Clinical diagnosis was performed by specialists in accordance with conventional standardized criteria and diagnostic procedures.

Clinical diagnosis – a single data point – was standardized according to widely accepted conventional criteria. These criteria were outlined both in the clinical trial protocol and the case report form and were adhered to at all sites.

Each subject underwent detailed neurological, neuropsychological, neuroradiological and other evaluations by experienced investigators at specialist clinics to determine clinical diagnosis. These evaluations conformed to the standards of NINCDS-ADRDA criteria and the American Academy of Neurology (AAN) Practice Guidelines (44-50). Evaluation in all probable or possible AD patients included medical history and examination, neurological examinations, psychosocial evaluation, neurocognitive assessment, Mini-Mental State Examination (MMSE), structural imaging study (CT or MRI or other scan), and blood tests (e.g., complete blood count, electrolytes, B12, thyroid function tests, liver function tests) to eliminate other known causes of cognitive impairment. Further tests such as EEG, CSF tests, genetic tests, functional imaging,

angiography, doppler studies, and further laboratory and other procedures were done in individual cases at the discretion of the individual Investigator (44-50).

The clinical trial protocol anticipated that, similar to the typical current clinical situation, the procedures performed for each subject would vary from case to case, depending upon case presentation and procedures already performed prior to referral, and would vary amongst different clinicians, depending upon the judgment of the physician in each individual case. For example, procedures already performed prior to referral might not be repeated if not judged necessary for diagnosis at the discretion of the investigator.

All AD cases had guideline workups, and 99% had at least one structural imaging study. Prior to diagnosis, the majority of cases were reviewed by diagnostic teams in consensus or other conferences at the sites. A majority of cases, depending upon the decision of the investigator, had further studies such as EEG, genetic testing, functional imaging (PET or FMRI or SPECT), angiography, doppler studies, CSF tests, or extended optional laboratory studies. The mean number of imaging studies per AD case was 1.32. Further, in response to FDA concerns (see Section D *FDA Issues* below), 17 cases (15%) had repeat CT or MRI scans so that all available consenting individuals had imaging studies within one year or less of the clinical diagnosis.

Patients were diagnostically categorized in the study as probable AD (46-48); possible AD (including mixed cases) (46-48); mild cognitive impairment (MCI) (45); or definite non-AD (including systemic, metabolic, infectious, hematologic, neurological, or neoplastic disease, depression, anxiety, pseudodementia, etc.).

B. Clinical Study Results

The results of the clinical trial for all 9 sites (N = 200) were as follows:

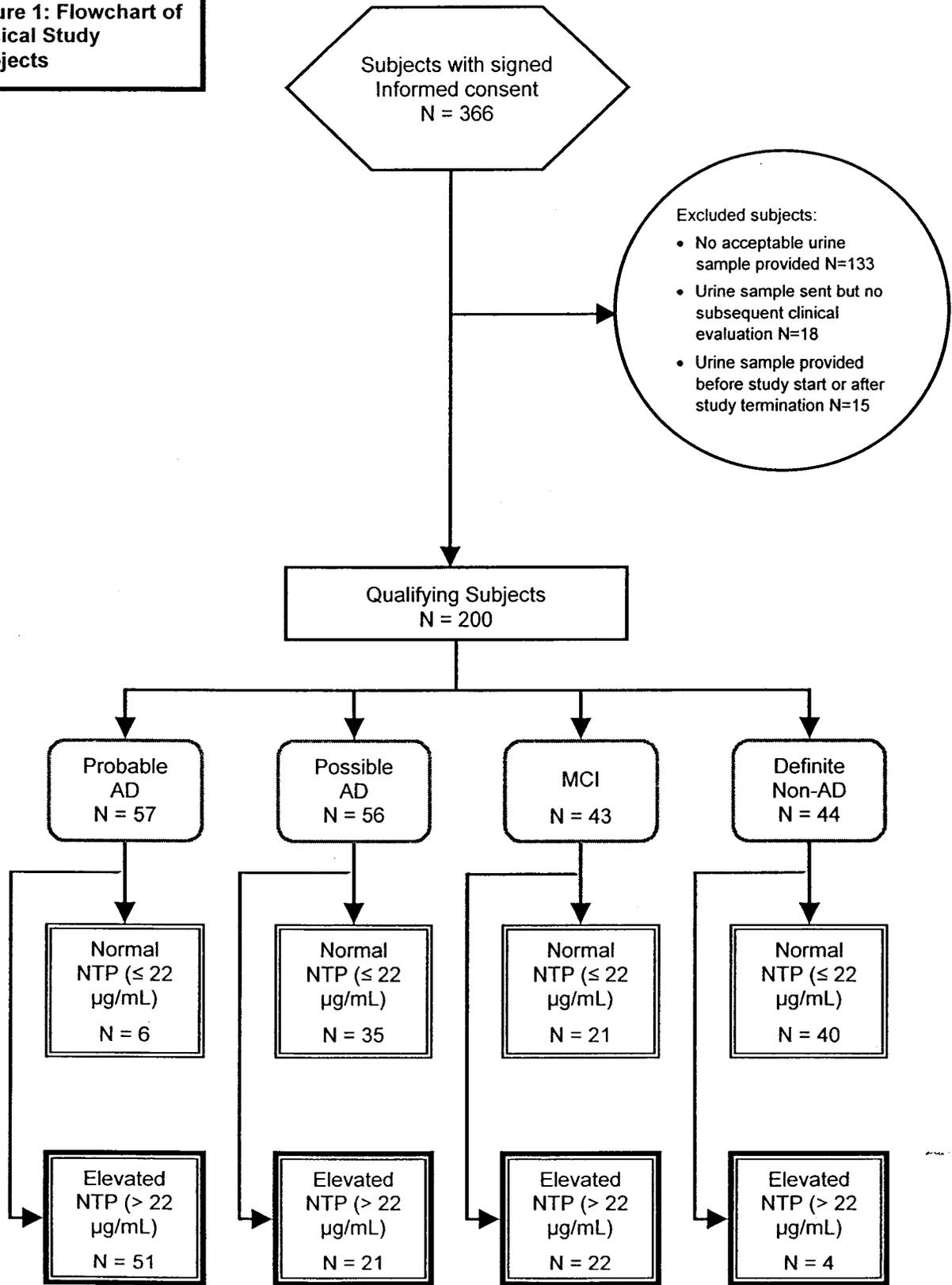
Table 1: Summary of Study Results

Cutoff = 22 µg/mL	Clinical Diagnosis Category				
	Probable AD	Possible AD	MCI	Definite Non-AD	Totals
NTP > 22	51	21	22	4	98
NTP ≤ 22	6	35	21	40	102
Totals	57	56	43	44	200

The resulting study population and its characteristics are quite typical of similar study populations in the literature (33-43). The prevalence of probable AD was 28.5%, possible AD 28.0%, MCI 21.5% and definite non-AD 22.0%.

Figure 1 depicts a flowchart of the subjects included and excluded in the clinical study.

Figure 1: Flowchart of Clinical Study Subjects



C. *Summary of Biostatistical Analysis of Results*

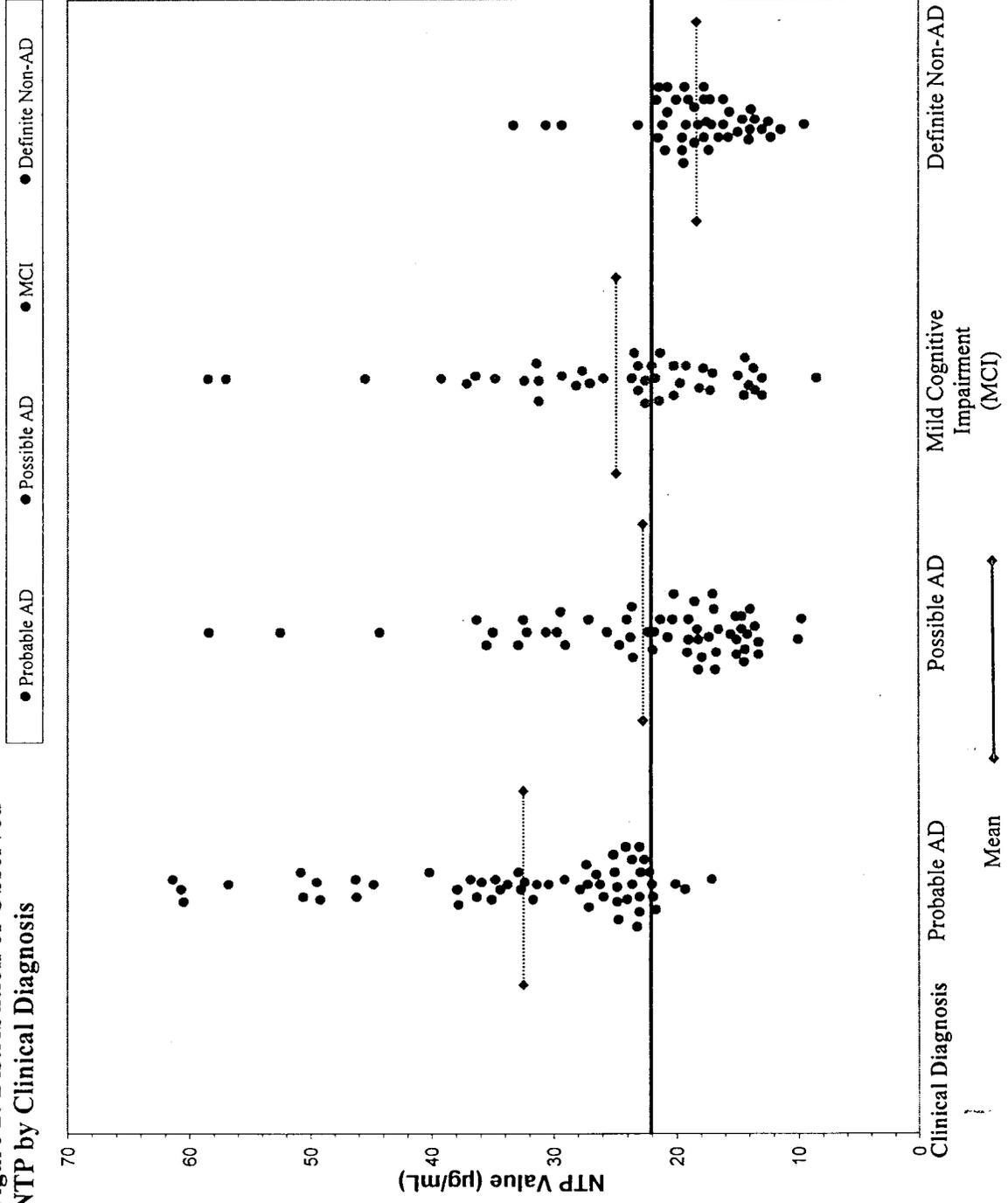
The overall results of the clinical study show the usefulness of NTP measurement.

The clinical study results show that NTP measurement provides useful diagnostic information to the diagnostic workup process. Figure 2 shows the distribution of NTP measurements for each of the 4 diagnostic groups and Table 2 the N, mean result, standard error of the mean, and 95% confidence interval on the mean for each group. The mean NTP measurement is essentially monotonically decreasing with decreasing likelihood of AD and the Kruskal-Wallis test shows a statistically significant ($p < 0.0001$) difference in the mean result between the groups. While there is some overlap in NTP results between diagnostic categories, there is a strong tendency for NTP measurements to be elevated in cases of probable Alzheimer’s disease as compared to NTP measurements in cases of possible AD, MCI, and definite non-AD and for NTP measurements to be normal in cases of definite Non-AD as compared to cases of probable AD, possible AD and MCI.

Table 2: Mean, Standard Error and Confidence Intervals for Observed NTP by Clinical Diagnosis

Diagnosis	Number	Mean	Std Error	Lower 95%	Upper 95%
Probable AD	57	32.4579	1.2894	29.915	35.001
Possible AD	56	22.4000	1.3009	19.834	24.966
Mild Cognitive Impairment (MCI)	43	24.7814	1.4846	21.854	27.709
Definite Non-AD	44	18.1932	1.4676	15.299	21.088
Total Number of Patients:	200				
Kruskal-Wallis test for median equality, chi squared statistic = 60.54 with 3 df, $p < 0.0001$					

Figure 2: Distribution of Observed NTP by Clinical Diagnosis



NTP measurement clearly distinguishes between Probable AD and Definite Non-AD at a cut-off of 22 µg/mL.

The clinical study results clearly demonstrate the ability of NTP measurement to discriminate between probable AD and definite non-AD using the device’s cut-off of 22 µg/mL. At this cut-off value, the sensitivity (% agreement) of NTP measurement for probable AD is 89.5% and the specificity (% agreement) of NTP measurement for definite non-AD is 90.9%. Consistent with clinical study design expectations, elevated NTP levels had intermediate percentage agreement with the diagnosis of Mild Cognitive Impairment (MCI) (51%) and Possible AD (37.5%).

The estimated predictive value of the device also demonstrates its utility in the clinical context. Table 3a below shows the clinical study results grouped as probable AD against not probable AD (possible AD, MCI and definite non-AD) and as definite non-AD against not definite non-AD (probable AD, possible AD and MCI). Table 3b shows the corresponding sensitivity, specificity, PPV and NPV values for these results. The PPV for an elevated NTP measurement to indicate probable or possible AD or MCI (i.e. not definite non-AD) is 95.9%. The NPV for a normal NTP measurement to indicate not probable AD (i.e. possible AD, MCI or definite non-AD) is 94.1%. The NPV for a normal NTP measurement to indicate definite non-AD is much lower (39.2%) but this nevertheless represents a 78% improvement over the 22% pre-test likelihood of that diagnosis in the study. Similarly the PPV for an elevated NTP measurement to indicate probable AD is 52.0% but again that represents an 82% improvement over the pre-test likelihood of probable AD in the study.

Table 3a: Clinical Study Results: Definite Non-AD vs. not Definite Non-AD; Probable AD vs. not Probable AD

Definite Non-AD	Not Definite Non-AD	Definite Non-AD	Totals	Probable AD	Probable AD	Not Probable AD	Totals
NTP > 22	94	4	98	NTP > 22	51	47	98
NTP ≤ 22	62	40	102	NTP ≤ 22	6	96	102
Totals	156	44	200	Totals	57	143	200

Table 3b: Estimated Sensitivity, Specificity, Positive and Negative Predictive Values for a Cut-off of 22 µg/mL

Definite Non-AD	% Result (95% C.I.)	Probable AD	% Result (95% C.I.)
Specificity (% of Def Non-AD that have Normal NTP)	90.9% (78.3% - 97.5%)	Sensitivity (% of Prob AD that have Elevated NTP)	89.5% (78.5% - 96.0%)
Sensitivity (% of not Def Non-AD [Prob AD + Poss AD + MCI] that have Elevated NTP)	60.3% (52.1% - 68.0%)	Specificity (% of not Prob AD [Poss AD + MCI + Def Non-AD] that have Normal NTP)	67.1% (58.8% - 74.8%)
Positive Predictive Value (PPV) (% of Elevated NTP that are not Def Non-AD)	95.9% (89.9% - 98.9%)	PPV (% of Elevated NTP that are Prob AD)	52.0% (41.7% - 62.2%)
PPV Improvement “with NTP” (compared to Prior Probability “without NTP”)	↑23%	PPV Improvement “with NTP” (compared to Prior Probability “without NTP”)	↑82%
Negative Predictive Value (NPV) (% of Normal NTP that are Def Non-AD)	39.2% (29.7% - 49.4%)	NPV (% of Normal NTP that are not Probable AD)	94.1% (87.6% - 97.8%)
NPV Improvement “with NTP” (compared to Prior Probability “without NTP”)	↑78%	NPV Improvement “with NTP” (compared to Prior Probability “without NTP”)	↑32%

NTP measurement shows utility for cut-off values ranging from 20 µg/mL to 30 µg/mL.

The clinical study results demonstrate that NTP measurement can provide useful diagnostic information for thresholds ranging from 20 µg/mL to 30 µg/mL. Based on the clinical study results, the optimal cut-off is 21.7 µg/mL. A cut-off value of 22 µg/mL is proposed for the labeling.

Table 4a sets out the clinical trial results for cut-offs of 20, 21.7, 22, 25 and 30 µg/mL and Table 4b the corresponding estimated sensitivities, specificities, and predictive values.

Table 4a: Results for Cut-off Values of 20, 21.7, 22, 25 and 30 µg/mL

Cut-off Threshold	Diagnosis					
		Prob AD	Poss AD	MCI	Def Non-AD	
20 µg/mL	NTP > 20	55	27	28	11	121
	NTP ≤ 20	2	29	15	33	79
	Totals	57	56	43	44	200
21.7 µg/mL	NTP > 21.7	53	23	23	4	103
	NTP ≤ 21.7	4	33	20	40	97
	Totals	57	56	43	44	200
22 µg/mL	NTP > 22	51	21	22	4	98
	NTP ≤ 22	6	35	21	40	102
	Totals	57	56	43	44	200
25 µg/mL	NTP > 25	36	15	16	3	70
	NTP ≤ 25	21	41	27	41	130
	Totals	57	56	43	44	200
30 µg/mL	NTP > 30	27	10	11	2	50
	NTP ≤ 30	30	46	32	42	150
	Totals	57	56	43	44	200

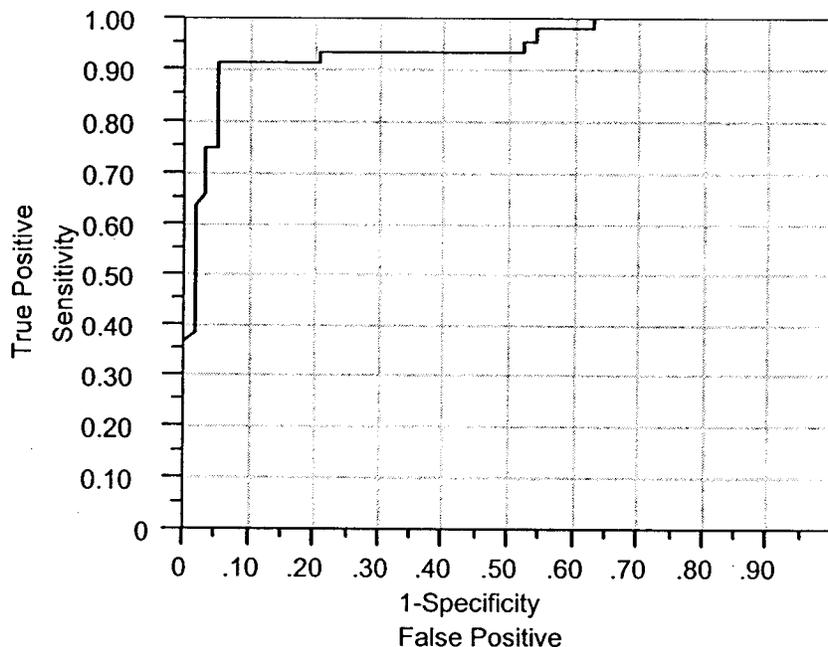
Table 4b: Estimated Sensitivity, Specificity, Positive and Negative Predictive Values for a Cut-off of 20, 21.7, 22, 25 and 30 µg/mL

	Cut-off Thresholds				
	20 µg/mL	21.7 µg/mL	22 µg/mL	25 µg/mL	30 µg/mL
Specificity (% of Def Non-AD that have Normal NTP)	75.00%	90.91%	90.91%	93.18%	95.45%
Sensitivity (% of not Def Non-AD [Prob AD + Poss AD + MCI] that have Elevated NTP)	70.51%	63.46%	60.26%	42.95%	30.77%
Positive Predictive Value (PPV) (% of Elevated NTP that are not Def Non-AD)	90.91%	96.12%	95.92%	95.71%	96.00%
PPV Improvement “with NTP” (compared to Prior Probability “without NTP”)	↑17%	↑23%	↑23%	↑23%	↑23%
Negative Predictive Value (NPV) (% of Normal NTP that are Def Non-AD)	41.77%	41.24%	39.22%	31.54%	28.00%
NPV Improvement “with NTP” (compared to Prior Probability “without NTP”)	↑90%	↑87%	↑78%	↑43%	↑27%
Sensitivity (% of Prob AD that have Elevated NTP)	96.49%	92.98%	89.47%	63.16%	47.37%
Specificity (% of not Prob AD [Poss AD + MCI + Def Non-AD] that have Normal NTP)	53.85%	65.03%	67.13%	76.22%	83.92%
PPV (% of Elevated NTP that are Prob AD)	45.45%	51.46%	52.04%	51.43%	54.00%
PPV Improvement “with NTP” (compared to Prior Probability “without NTP”)	↑59%	↑81%	↑82%	↑80%	↑89%
NPV (% of Normal NTP that are not Prob AD)	97.47%	95.88%	94.12%	83.85%	80.00%
NPV Improvement “with NTP” (compared to Prior Probability “without NTP”)	↑36%	↑34%	↑32%	↑17%	↑12%

NTP measurement shows utility in clearly distinguishing probable AD from definite non-AD.

An analysis was conducted of the ability of NTP measurement to distinguish probable AD from definite non-AD. Figure 3 below shows the ROC (receiver operator characteristic) curve for probable AD against definite non-AD. The curve exhibits a healthy 0.94 area under the curve (AUC).

Figure 3: Discrimination prognosis: Probable AD vs. Non-AD



Area under curve (AUC) = 0.9398

The odds ratio calculation for probable AD against definite non-AD is set out in Table 5 below along with corresponding 95% confidence intervals. The odds ratio is a measure of the likelihood (or probability) that a patient belongs to the probable AD classification as opposed to the likelihood that a patient belongs in the definite non-AD group. If the 95% confidence interval for the odds ratio lies completely above 1.00, the test demonstrates a significant ability to determine if a given patient is at higher or lower risk of having AD. Here the 95% confidence interval lies well above the 1.00, further indicating the value of NTP measurement in the AD diagnostic work-up.

**Table 5: Risk Assessment by Odds Ratio Analysis
 Discrimination: Probable AD vs. Non-AD
 NTP Thresholds of 22 µg/mL**

	Probable AD	Definite Non-AD	Totals
NTP > 22	51	4	55
NTP ≤ 22	6	40	46
Totals	57	44	101

Odds of AD NTP>22	12.75
Odds of AD NTP≤22	0.15
Odds Ratio (OR)	85.0
95% LOWER CI limit	22.4
95% UPPER CI limit	321.8

The clinical study results showed no effect due to age or gender.

Statistical analysis of the clinical study results showed no effect due to age or gender.

There was no relationship between age and NTP results. Figure 4 plots age against NTP measurement (for all 4 diagnostic categories combined) and Figures 4a-d for each of the 4 diagnostic categories separately. In Figure 4, the R² value of variance explained by age is only 0.025, confirming the visual interpretation of no relationship. Visual examination of each of the individual category scatterplots and corresponding R² values demonstrate this lack of effect of age within each diagnostic group as well.

Figure 4: Bivariate Scatterplot of NTP Results by Patient Age (All 4 Diagnostic Groups Combined)

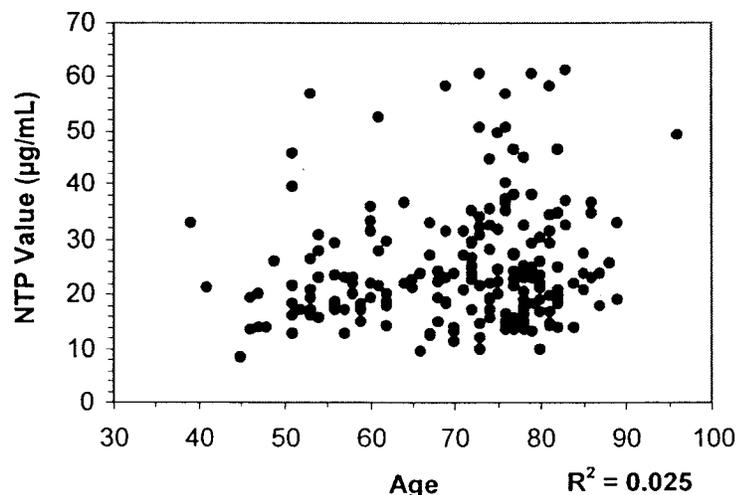


Figure 4a: NTP Distribution by Age for Probable AD

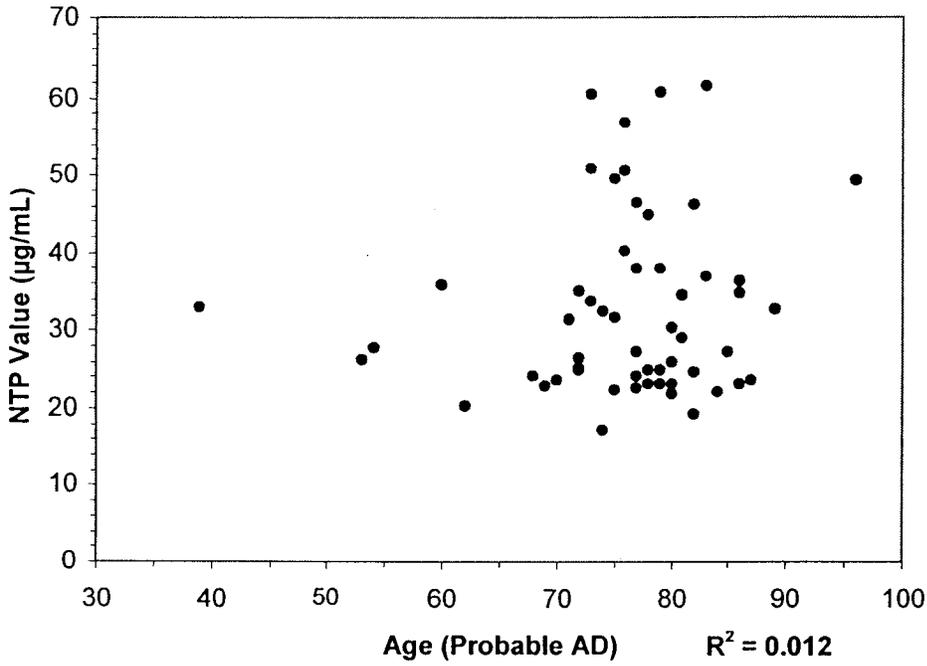


Figure 4b: NTP Distribution by Age for Possible AD

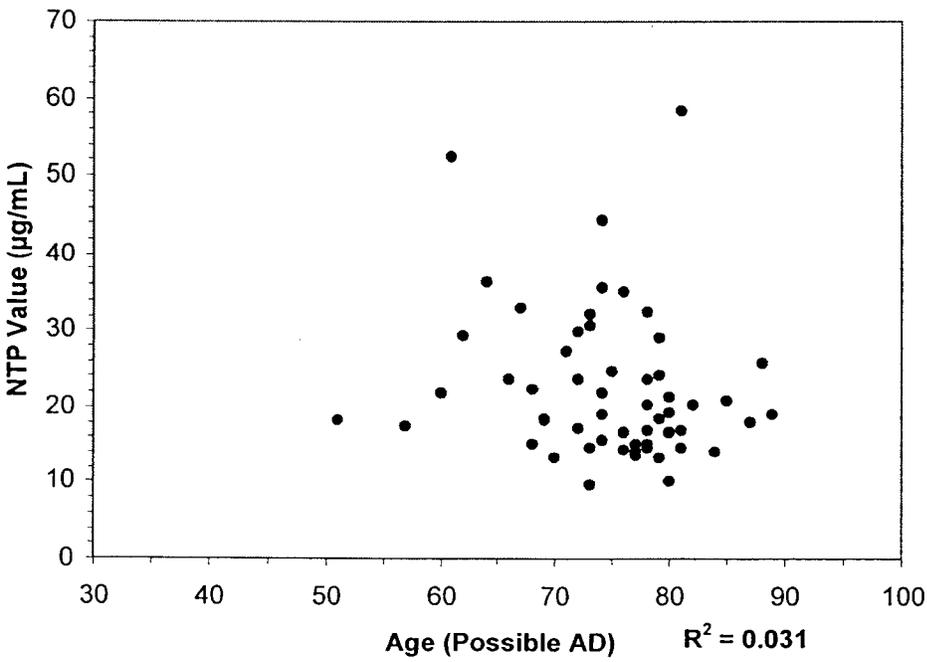


Figure 4c: NTP Distribution of Age for MCI

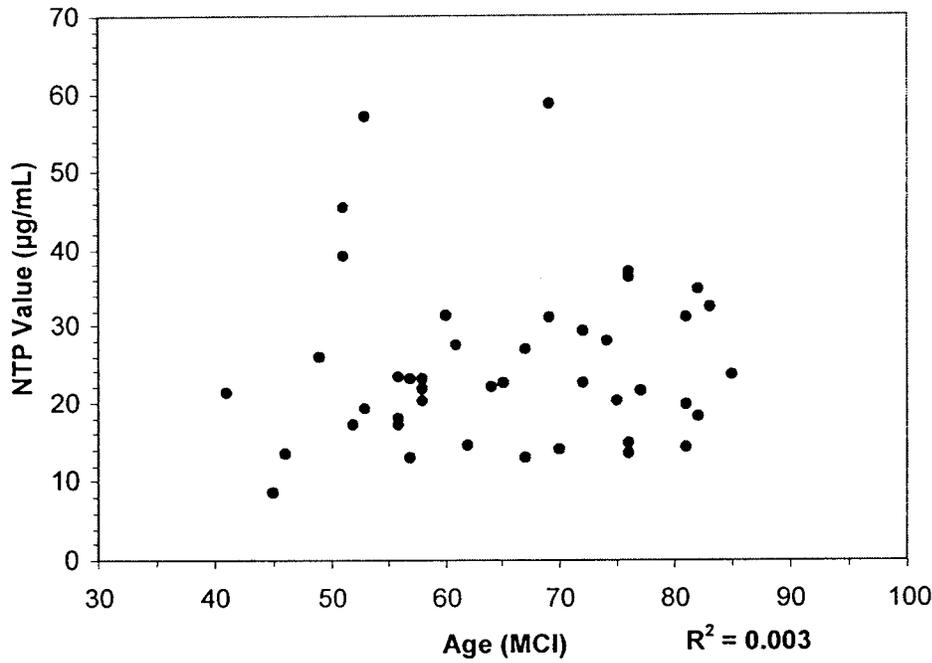
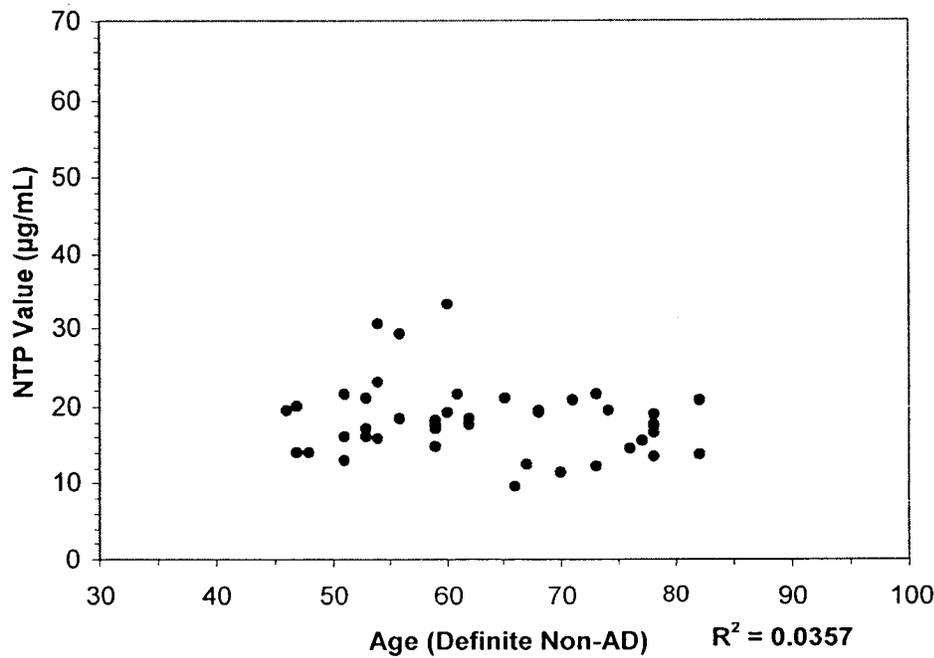


Figure 4d: NTP Distribution by Age for Definite Non-AD



There were no significant differences noted in gender split between the 4 diagnostics groups (p=0.47 by chi-squared contingency table analysis) nor in the distribution of NTP assay results (p=0.75 by Wilcoxon test). Table 6 shows the distribution of patient gender by diagnosis. Figure 5 shows the distribution of the NTP assay results by gender over all 4 groups.

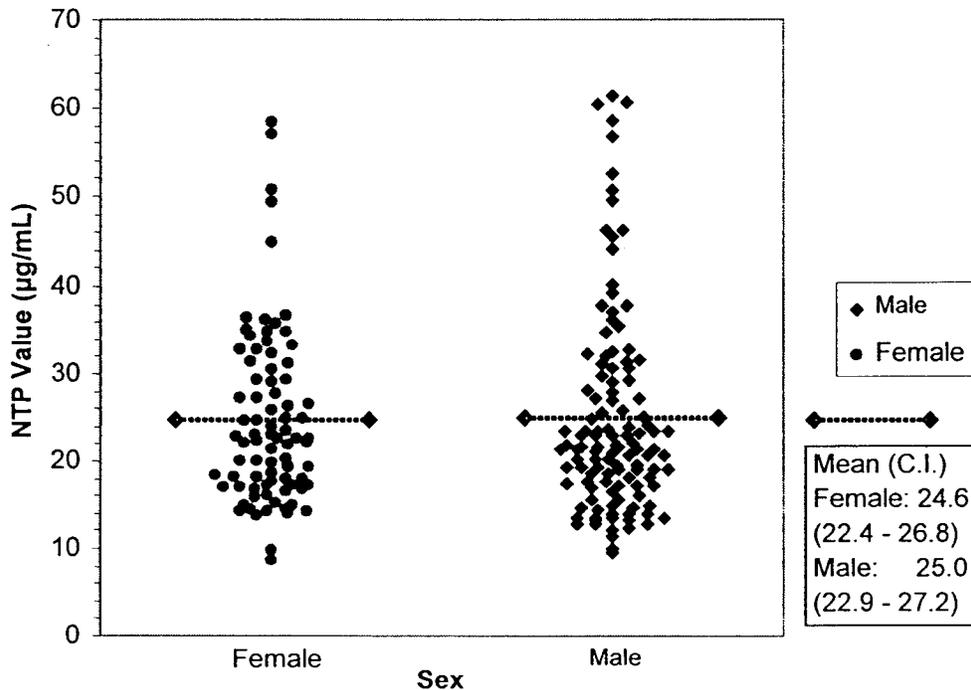
Table 6: Distribution of Patient Genders by Diagnosis

Diagnosis	Males	Females
Probable AD	30 (53%)	27 (47%)
Possible AD	33 (59%)	23 (41%)
Mild Cognitive Impairment (MCI)	25 (58%)	18 (42%)
Definite Non-AD	30 (68%)	14 (32%)

Likelihood Ratio chi-squared p-value = 0.47

No significant differences noted in gender distributions between diagnostic categories.

Figure 5: Distribution of Observed NTP by Gender

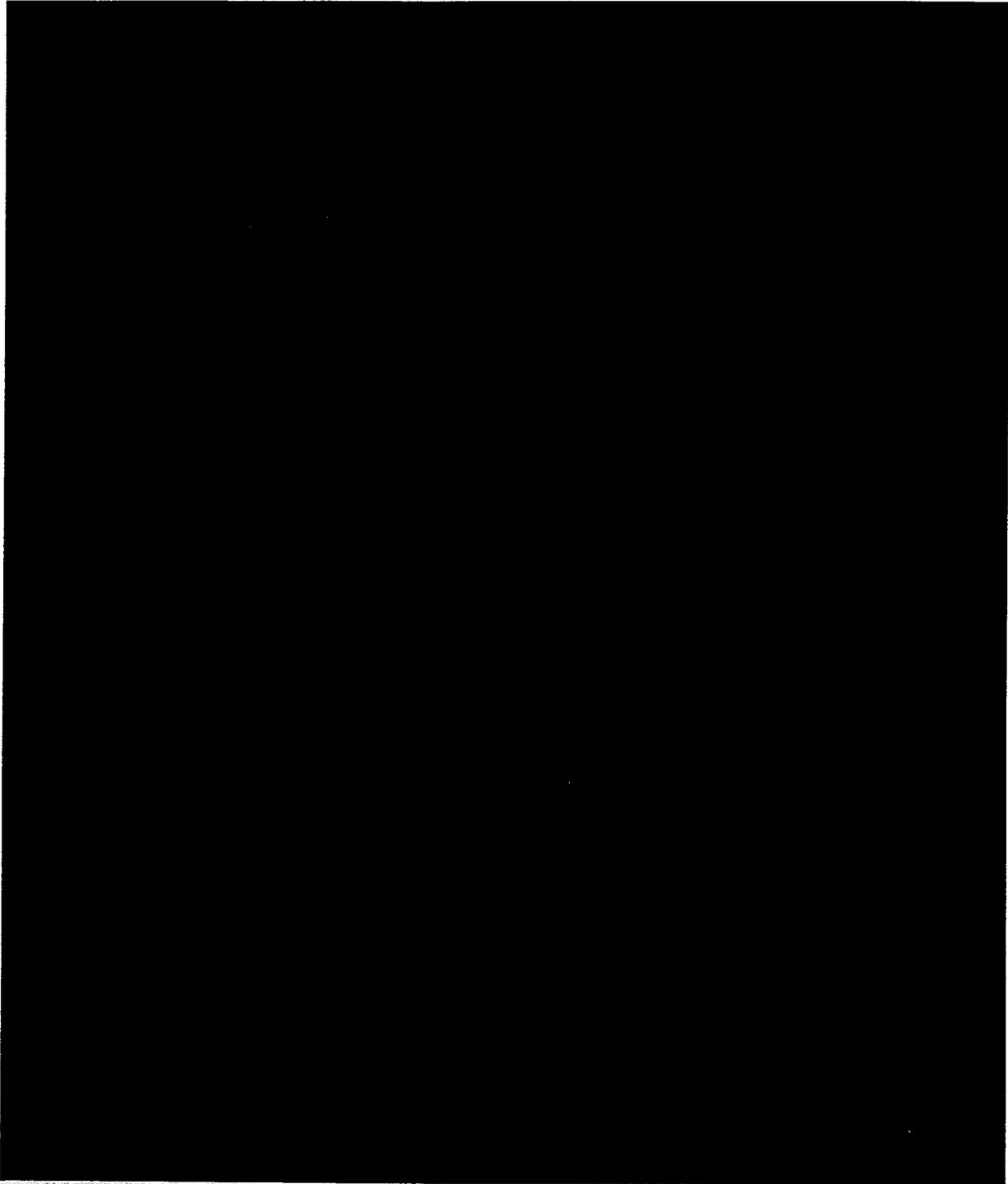


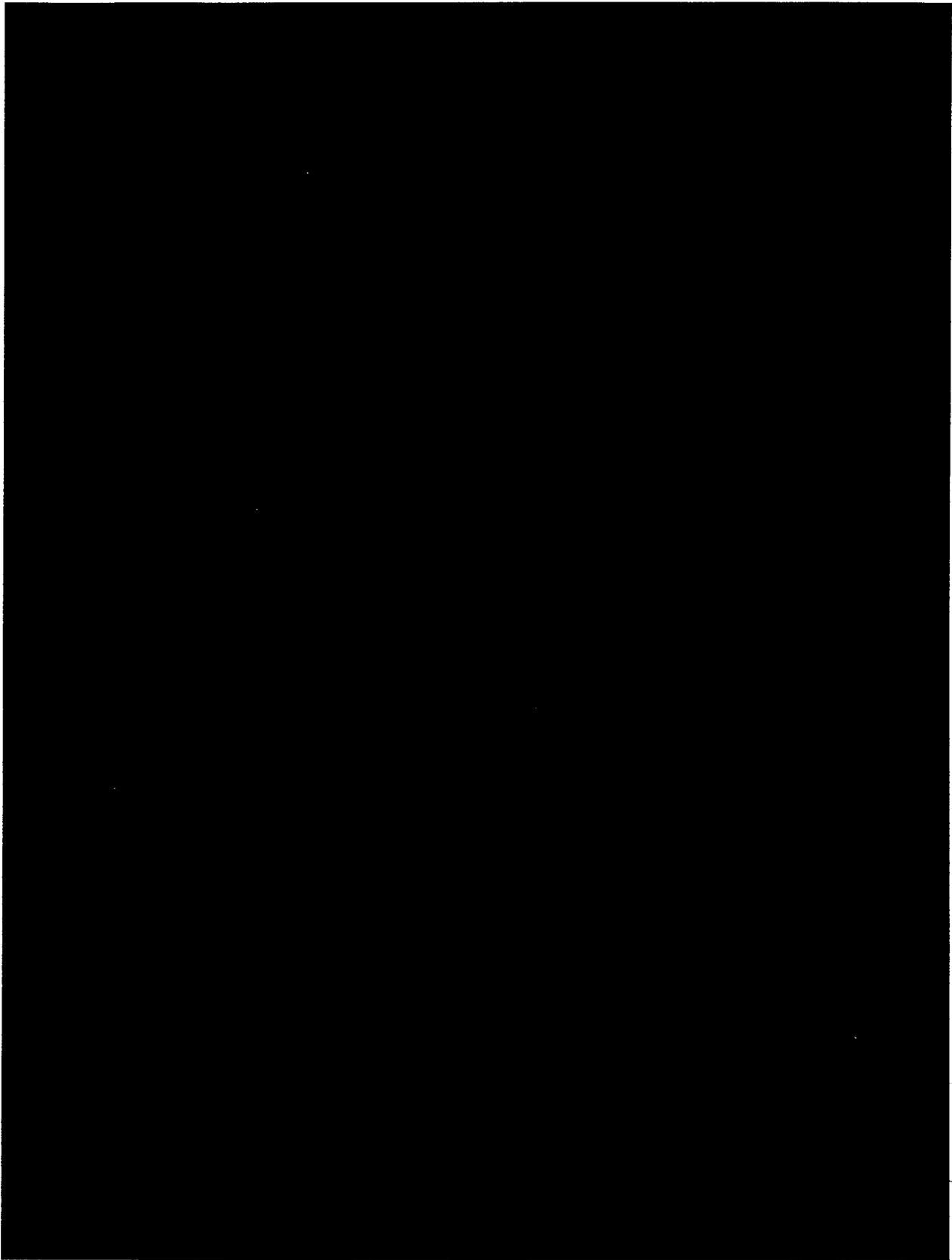
No significant differences found in NTP distribution by gender (Wilcoxon test p-value-0.75).

Conclusion: The clinical study results show that NTP measurement by the urine NTP assay adds useful information to the AD diagnostic process.

The clinical study results show that NTP measurement provides useful diagnostic information to the diagnostic workup process for suspected AD. The mean NTP measurement for probable AD patients (32.5 $\mu\text{g/mL}$) was significantly greater ($p < 0.0001$; Kruskal-Wallis) than the mean values for definite non-AD (18.1 $\mu\text{g/mL}$) and for possible AD (22.4 $\mu\text{g/mL}$) and MCI (24.8 $\mu\text{g/mL}$). Specificity (% agreement) of a normal NTP measurement for definite non-AD was high (90.9%) as was the sensitivity (% agreement) of an elevated NTP measurement for probable AD (89.5%). Positive predictive value (PPV) of an elevated NTP measurement for subjects who were diagnosed as probable AD or possible AD or MCI (i.e. not definite non-AD) was also high (95.9%). NTP measurement significantly improved NPV (percentage of normal NTP that are definite non-AD) as compared to prior probability for definite non-AD (78% improvement), and significantly improved PPV as compared to prior probability for not definite non-AD (23% improvement).







IV. Summary of Non-Clinical Studies

1. 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, biaxin, fosamax, lipitor, losec, acetaminophen, ibuprofen, potassium, pepcid, indur, temazepam.

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

2. 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 measurements of any of the urine samples.

3. 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.

4. 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%.

5. Analytical Threshold

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

6. 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).

7. Stability Studies

Stability studies indicate that refrigerated coated plates are stable for 8 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 ≥ 3 months.

8. Freeze-Thaw of Processed Urine

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).

9. Lot to Lot Variation

Standards from 2 lots were tested in 232 replicates on 29 different days, and the CVs within lots were 4.0-4.4% (10 µg/mL), 3.0-4.4% (20 µg/mL), 5.2-5.6% (40 µg/mL), and 7.8-12.1% (80 µg/mL). Standards from 3 lots were tested on 3 consecutive days in 9 replicates, with CVs of 1.3% (10 µg/mL), 1.8% (20 µg/mL), 3.0% (40 µg/mL), and 10.2% (80 µg/mL). Urine high, medium, and low-NTP controls were tested for 3 days in 108 replicates, with CVs of 8.6% (low-NTP), 4.3% (medium-NTP), and 6.3% (high-NTP). Coated plates from 3 lots were compared on 3 different days in 45 replicates using 5 standards, with overall CVs of 0.8% (0 µg/mL), 1.8% (10 µg/mL), 2.4% (20 µg/mL), 3.2% (40 µg/mL), and 14.2% (80 µg/mL).

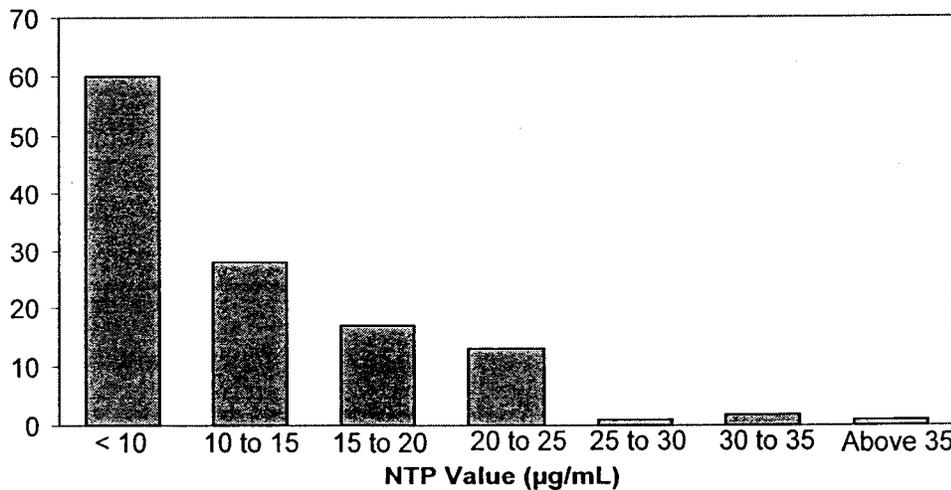
10. Transport of Kit

Urine NTP kits in styrofoam boxes (frozen test kit components shipped with dry ice, and refrigerated components with cool packs) 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 measurement results.

11. Normal Reference Range Study

122 normal individuals (with no history of cognitive complaints, symptoms, or disorders) were assayed. The distribution of results is shown in Figure 6. Ninety percent of reference individuals have urine NTP ≤22 µg/mL. There were no significant differences according to age or gender.

Figure 6: Distribution of Observed NTP in 122 Normal Individuals



Percentiles		
100.0%	maximum	36.70
99.5%		36.70
97.5%		32.16
90.0%		22.16
75.0%	quartile	16.33
50.0%	median	10.00
25.0%	quartile	9.50

Gender	Number	Mean	Std Error	Lower 95%	Upper 95%
F	56	13.1993	0.76681	11.681	14.718
M	65	13.4882	0.71174	12.079	14.897

t-test p-value = 0.78 (no significant difference)
 Wilcoxon test p-value = 0.93 (no significant difference)

V. Scientific Background of NTP Biomarker (References 8-32 and Attached Reprints, *J Clin Invest* 1997; 100: 3093-3104, and *J. Neuropathol Exp Neurol* 1996; 55:1038-1050)

The pathophysiological relevance of NTP to AD is based on 1) immunohistochemical studies of postmortem AD brains; 2) experimental in vitro studies; and 3) various research assays of NTP in postmortem AD brain, antemortem CSF, and urine.

cDNA was isolated from a library prepared with temporal lobe mRNA from a brain with definite AD, and the recombinant expressed protein was used to generate monoclonal antibodies (8-10). These antibodies to the recombinant putative NTP (anti-NTP mAbs) were used in immunohistochemical studies of paraffin sections of Brodmann Areas 11 (frontal) and 21 (temporal) from AD and control brains (10). Immunoreactivity was localized in neuronal perikarya, neuropil fibers, and white matter fibers, and AD brains had increased intensity of immunoreactivity and higher percentage of labeled cerebral cortical cells. Immunoreactivity was most prominent in neurons cytologically intact or slightly degenerated, with or without intracellular neurofibrillary tangles (NFT), as contrasted with non-immunoreactive apoptotic cells and extracellular NFT. The anti-NTP mAbs labeled cortical neurons, neuropil, swollen axons, dystrophic neurites, protoplasmic astrocytes, oligodendrocytes, as well as intracellular NFT and degenerated neurons without NFT (9, 10).

Double-labeling studies with anti-NTP and anti-A β showed dual participation (without co-localization) in diffuse plaques. In contrast, in dense cored plaques there was no NTP immunoreactivity, although nearby neurons were strongly positive. NTP immunoreactivity co-localized with phospho-tau, phosphorylated high and middle-molecular weight neurofilament, and A $_2$ B $_5$ in neuronal perikarya, abnormal neurites, and swollen axons (9, 10). Findings in Down syndrome (where there is early onset AD) postmortem brains also suggest that the immunoreactivity occurs early in the course of AD neurodegeneration (18). Overall, increased expression of NTP immunoreactivity is detectable early and in histologically normal-appearing neurons. With increasing neuronal degeneration, NTP and phospho-tau immunoreactivity increase, but NTP becomes undetectable in late-stage and extracellular NFT (29).

Transfection of human neuronal cells in culture with the putative NTP cDNA leads to 1) cell death, and 2) prominent neuritic sprouting (8, 32). Overexpression in vitro causes neuronal cell death mediated by apoptosis and impaired mitochondrial function, and activation of the pro-apoptotic pathways observed in AD brains (12). The remaining cells in culture exhibit prominent neuritic sprouting with increased expression of synaptophysin and phospho-tau (12). It has been suggested that the NTP over-expression may lead to increased cellular sensitivity to oxidative stress and free radical injury, or it may promote a pro-apoptotic or sprouting response (29).

The corresponding mRNA expression was examined in postmortem AD brain by Northern blot analysis and in situ hybridization (8). Radiolabeled cDNA probes detected the mRNA transcripts in brain but not in pancreas, intestines, liver, lung, ovary, and testis. There was statistically significantly higher mean levels of the transcripts in a series of AD brains compared to aged non-AD control brains. In situ hybridization also showed higher levels of NTP-related expression in AD brain in comparison to aged control brain (8).

The anti-NTP mAbs were used in Western blot analyses and sandwich assays. Western blots showed the putative NTP bands in postmortem AD brains, and densitometric measurements demonstrated significantly higher levels in a series of postmortem AD brain samples, in comparison to aged control samples (10). Western blots also showed the same positive bands, and higher levels in postmortem

ventricular fluid from definite AD, compared to age-matched control samples. An enzyme-linked sandwich immunosorbant assay study of postmortem brain tissue homogenates showed significantly higher NTP in postmortem AD brain samples compared to age-matched control samples (8, 10). The same assay showed significantly higher levels in post-mortem AD ventricular fluid compared to age-matched control samples (8). Antemortem lumbar CSF samples from over 300 AD cases and controls were studied with the same anti-NTP mAb based assay (8, 20, 23) which showed 1) nearly 3X the mean level in AD CSF compared to controls, 2) 89% sensitivity and 89% specificity of elevated NTP for AD versus a age-matched control cases, and 3) significant correlation between Blessed dementia scores and assay levels, but not between assay level and age or gender (8). Similar significant results were also found using the same anti-NTP mAb based sandwich assay on 24-hour antemortem urine samples from AD cases and controls (25).

According to the well documented and very careful studies in the published literature, the cDNA derived from human AD brain characterized above is believed to contain 1442 base pairs with a translated putative 375 amino acid sequence predicted which contains several Alu-type sequences, as well as a predicted membrane-spanning region, and possible 17 cAMP, calmodulin-dependent protein kinase II, phosphorylation and myristoylation sites, and a region with homology to the IGF1-insulin receptor (8, 19, 29). The final consensus exact structure and location of the human NTP/NTP related gene(s) however are still under investigation.

Current basic scientific research includes studies of experimental animals with NTP related gene transfer, where it has been reported that animals exhibit some changes in behavior, as well as intracerebral neuronal cell loss, amyloid deposits, and phospho-tau production. It has been suggested that the above changes may also provide a compelling demonstration of relevance to AD, and may be useful as a practical research model for assessment of AD therapeutics (65). The role of NTP-related molecules(s) in AD-related oxidative and free radical injury is also currently under investigation, in addition to various pathophysiological, genetic, analytical, and other studies in progress (29, 65-67).

VI. References

1. Callahan CM, Hendric HC, Tierney WM. Documentation and evaluation of cognitive impairment in elderly primary care patients. *Am Int Med* 1995; 122:422-429.
2. Ross GW, Abbott RD, Petrovich H, *et al.* Frequency and characteristics of silent dementia among elderly Japanese-American men: the Honolulu-Asia Aging Study. *JAMA* 1997; 277:800-805.
3. Valcour VG, Masaki KH, Curb JD, Blanchette PL. The detection of dementia in the primary care setting. *Arch Int Med* 2000; 160: 2964-2968.
4. Williamson J, Stokoe IH, Gray S, Fisher M, Smith A, McGhee A, Stephenson E. Old people at home: their unreported needs. *Lancet* 1964; i: 1117-1120.
5. Wind AW, Van Staveren G, Schellevis FG, Jonker C, Van Eyk JTM. The validity of the judgement of general practitioners on dementia. *International Journal of Geriatric Psychiatry* 1994; 9: 543-549.
6. Sternberg SA, Wolfson C, Baumgarten M. Undetected dementia in community-dwelling older people: the Canadian Study of Health and Aging. *J Am Geriatr Soc* 2000; 48: 1430-1434.
7. Boise L, Camicioli R, Morgan DL, *et al.* Diagnosing dementia: Perspectives of primary care physicians. *The Gerontologist* 1999; 39: 457-464.
8. de la Monte, SM, *et al.* Characterization of the AD7C-NTP cDNA expression in Alzheimer's disease and measurement of a 41-kD protein in cerebrospinal fluid. *J Clin Invest* 1997; 100: 3093-3104.

9. de la Monte SM, Xu YY, Wands JR. Modulation of neuronal thread protein expression with neuritic sprouting: relevance to Alzheimer's disease. *J Neurol Sci* 1996; 138: 26-35.
10. de la Monte SM, Carlson RI, Brown NV, Wands JR. Profiles of neuronal thread protein expression in Alzheimer's disease. *J Neuropathol Exp Neurol* 1996; 55: 1038-1050.
11. de la Monte SM, Wands JR. Neural thread protein over-expression in brains with Alzheimer's disease lesions. *J Neurol Sci* 1992; 113: 152-64.
12. de la Monte SM, Wands JR. Alzheimer-associated neuronal thread protein-induced apoptosis and impaired mitochondrial function in human central nervous system-derived neuronal cells. *J Neuropathol Exp Neurol* 2001; 60 (2): 195-207.
13. de la Monte SM, Ozturk M, Wands JR. Enhanced expression of an exocrine pancreatic protein in Alzheimer's disease and the developing human brain. *J Clin Invest* 1990; 86: 1004-13.
14. Ozturk M, de la Monte SM, Gross J, Wands JR. Elevated levels of an exocrine pancreatic secretory protein in Alzheimer's disease brain. *Proc Natl Acad Sci USA* 1989; 86: 419-23.
15. de la Monte SM, Volicer L, Hauser SL, Wands JR. Increased levels of neuronal thread protein in cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol* 1992; 32: 733-42.
16. Xu YY, Bhavani K, Wands JR, de la Monte SM. Ethanol inhibits insulin receptor substrate-1 tyrosine phosphorylation and insulin-stimulated neuronal thread protein gene expression. *Biochem J* 1995; 310: 125-32.
17. Xu YY, Bhavani K, Wands JR, de la Monte SM. Insulin induced differentiation and modulation of neuronal thread protein expression in primitive neuroectodermal tumor cells is linked to phosphorylation of insulin receptor substrate-1. *J Mol Neurosci* 1995; 6: 91-108.
18. de la Monte SM, Xu YY, Hutchins GM, Wands JR. Developmental patterns of neuronal thread protein gene expression in Down syndrome. *J Neurol Sci* 1996; 135: 118-25.
19. de la Monte S, et al. AD7C-NTP biomarker for Alzheimer's disease. *Alzheimer's Reports* 1999; 2(6): 327-332.
20. Ghanbari H, et al. Specificity of AD7C-NTP as a Biochemical Marker for Alzheimer's Disease. *J Contemp Neurol* 1998; 4: 2-6.
21. Ghanbari K, Ghanbari H. A Sandwich Enzyme Immunoassay for Measuring AD7C-NTP as an Alzheimer's Disease Marker: AD7C Test. *J Clin Lab Anal* 1998; 12: 223-226.
22. Chong JK, et al. Automated microparticle enzyme immunoassay for neural thread protein in cerebrospinal fluid from Alzheimer's disease patients. *J Clin Lab Anal* 1992; 6: 379-83.
23. Kahle PJ, Jakowec M, Teipel SJ, et al. Combined assessment of Tau and neuronal thread protein in Alzheimer's disease CSF. *Neurology* 2000; 54: 1498-1504.
24. Fitzpatrick J, et al. 7C Gold urinary assay of neural thread protein in Alzheimer's disease. *Alzheimer's Reports* 2000; 3(3): 155-159.
25. Ghanbari H, et al. Biochemical Assay for AD7C-NTP in Urine as an Alzheimer's Disease Marker. *J Clin Lab Anal* 1998; 12: 285-288.
26. Xu YY, Wands JR, de la Monte SM. Characterization of thread proteins expressed in neuroectodermal tumors. *Cancer Res* 1993; 53: 3823-9.
27. Munzar M, Levy S, Rush R, et al. Competitive ELISA format urinary assay of neural thread protein in Alzheimer's disease. *Alzheimer's Reports* 2001; 4 (2): 61-65.
28. Munzar M, McConville M, Yung J, et al. A retrospective clinical study of urinary neural thread protein in Alzheimer's disease. *Alzheimer's Reports* 2002; 5(1): 1-6.

29. de la Monte SM, Wands JR. The AD7c-NTP neuronal thread protein biomarker for detecting Alzheimer's disease. *J. Alz. Dis.* 2001; 3: 345-353.
30. Munzar M, Levy S, *et al.* Clinical study of a urinary competitive ELISA for neural thread protein in Alzheimer's disease. *Neurol. Clin. Neurophysiol.* 2002; 1, 2-7.
31. Ghanbari, H, *et al.* Specificity of AD7C-NTP as a biochemical marker for Alzheimer's disease. *Neurol. Clin. Neurophysiol.* 1998; 4: 2-6.
32. de la Monte SM, Wands JR. Neurodegeneration changes in primary central nervous system neurons transfected with the Alzheimer-associated neuronal thread protein gene. *Cell, Mol, Life Sci*, 2001; 58: 844-849.
33. Larson EB, Reifler BV, *et al.* Diagnostic Tests in the Evaluation of Dementia: A Prospective Study of 200 Elderly Outpatients. *Arch Intern Med* 1986; 146: 1917-1922.
34. Larson EB, Reifler BV, *et al.* Dementia in Elderly Outpatients: A Prospective Study. *Ann. Int. Med.* 1984; 100: 417-423.
35. Becker JT, Boller F, *et al.* The Natural History of Alzheimer's Disease: Description of Study Cohort and Accuracy of Diagnosis. *Arch. Neurol.* 1994; 51: 585-594.
36. Folstein MF, Bassett SS, *et al.* Dementia: Case Ascertainment in a Community Survey. *J. Gerontol.* 1991; 46: M132-138.
37. Freter S, Bergman H, *et al.* Prevalance of potentially reversible dementias and actual reversibility in a memory clinic cohort. *CMAJ* 1998; 159: 657-62.
38. Gurland BJ, Wilder DE, *et al.* Rates of Dementia in Three Ethnoracial Groups. *Int. J. Geriat. Psychiatry* 1999; 14: 481-493.
39. Ames D, Flicker L, Helme RD. A memory clinic at a geriatric hospital: rationale, routine and results from the first 100 patients. *Med. J. Aust.* 1992; 156: 618-622.
40. Evans DA, Funkenstein HH, *et al.* Prevalence of Alzheimer's Disease in a Community Population of Older Persons: Higher Than Previously Reported. *JAMA* 1989; 262: 2551-2556.
41. Larson EB, Reifler BV, *et al.* Diagnostic Evaluation of 200 Elderly Outpatients with Suspected Dementia. *J. Gerontol.* 1985; 40: 536-543.
42. Breitner JCS, Wyse BW, *et al.* APOE- ϵ 4 count predicts age when prevalence of AD increases, then declines: The Cache County Study. *Neurology* 1999; 53: 321-331.
43. Massoud F, Devi G, *et al.* The Role of Routine Laboratory Studies and Neuroimaging in the Diagnosis of Dementia: A Clinicopathological Study. *J Am Geriatr Soc* 2000; 48: 1204-1210.
44. Knopman, D.S., DeKosky, S.T., Cummings, J.L., *et al.* Practice parameter: Diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2001; 56: 1143-1153.
45. Peterson RC, Stevens JC, Ganguli M, *et al.* Practice parameter: Early detection of dementia: Mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2001; 56: 1133-1142.
46. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan E. 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. *Neurology* 1984; 34: 939-44.
47. Report of the Quality Standards Subcommittee of the American Academy of Neurology. Practice parameters for diagnosis and evaluation of dementia (Summary statement). *Neurology* 1994; 44: 2203-2206.

48. Tierney MC, Fisher RH, Lewis AJ, Zorzitto ML, Snow WG, Reid DW. The NINCDS-ADRDA Work Group criteria for the clinical diagnosis of probable Alzheimer's disease: a clinico-pathologic study of 57 cases. *Neurology* 1988; 38: 359-364.
49. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders, 4th edn.* Washington, DC: American Psychiatric Association, 1994.
50. Chui H, Zhang Q. Evaluation of dementia: a systematic study of the usefulness of the American Academy of Neurology practice parameters. *Neurology* 1997; 49: 925-35.
51. Scheltens P, Fox N, et al. Structural magnetic resonance imaging in the practical assessment of dementia: beyond exclusion. *Lancet Neurology* 2002; 1: 13-21.
52. Perl DP, Olanow CW, Calne D. Alzheimer's disease and Parkinson's disease: distinct entities or extremes of a spectrum of neurodegeneration? *Ann Neurol* 1998; 44 (suppl 1): S19-S31.
53. Hakim AM, Mathieson G. Dementia in Parkinson disease: a neuropathologic study. *Neurology* 1979; 29: 1209-1214.
54. Boller F, Mizutani T, Roessmann U, Gambetti P. Parkinson disease, dementia and Alzheimer disease: clinicopathological correlations. *Ann Neurol* 1980; 7: 329-335.
55. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinicopathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992; 55: 181-184.
56. Lopez OL, Becker JT, et al. Research evaluation and prospective diagnosis of dementia with Lewy bodies. *Arch Neurol* 2002; 59: 43-46.
57. Hohl, U, Tiraboschi P, et al. Diagnostic accuracy of dementia with Lewy bodies. *Arch Neurol* 2000; 57: 347-351.
58. Holmes C, Cairns N, et al. Validity of current clinical criteria for Alzheimer's disease, vascular dementia and dementia with Lewy bodies. *Br J Psych* 1999; 174: 45-50.
59. Hansen LA, Samuel W. Criteria for Alzheimer's disease and the nosology of dementia with Lewy bodies. *Neurology* 1997; 48: 126-132.
60. Perry RH, Irving D, Blessed G, Fairbairn A, Perry EK. Senile dementia of the Lewy body type: a clinical and neuropathologically distinct form of Lewy body dementia in the elderly. *J Neurol Sci* 1990; 95: 119-139.
61. Kosaka K. Diffuse Lewy body disease in Japan. *J Neurol* 1990; 237: 197-204.
62. Ince P, Irving D, MacArthur F, Ferry RH. Quantitative neuropathological study of Alzheimer-type pathology in the hippocampus: comparison of senile dementia of Lewy body type, Parkinson's disease and non-demented elderly control patients. *J Neurol Sci* 1991; 106: 142-152.
63. Bergeron C, Pollanen M. Lewy bodies in AD: one or two diseases? *Alzheimer Dis Assoc Disord* 1989; 3: 197-204.
64. Bierer LM, Perl DP, Haroutunian V. Neurofibrillary tangles, Alzheimer's disease, and Lewy bodies. *Lancet* 1990; 335: 8682.
65. Wands, J. et al. Non-transgenic nonhuman model for Alzheimer's disease using AD7c-NTP nucleic acid. United States Patent 6,770,797; August 3, 2004.
66. de la Monte SM, Chen GJ, et al. Neuronal thread protein regulation and interaction with microtubule-associated proteins in SH-Sy5y neuronal cells. *CMLS Cell Mol Life Sci* 2003; 60: 2679-2691.
67. de la Monte SM, Wands JR. Alzheimer-associated neuronal thread protein mediated cell death is linked to impaired insulin signaling. *J. Alz. Dis.* 2004; 6: 231-242.

VII. Speakers Representing Sponsor at Advisory Panel Meeting

Speakers representing Nymox include:

Ira Goodman MD [REDACTED], Stephen Flitman MD [REDACTED]

Patricio Reyes MD [REDACTED]

Daniel A. Bloch PhD [REDACTED]

Paul Averback MD, DABP (Neuropath) [REDACTED]

Susanna Levy PhD [REDACTED]

VIII. Proposed Labeling

Instructions for Use: Urine NTP Kit

URINE NTP KIT

Microtiter ELISA format assay kit for laboratory use. It provides sufficient materials to perform 8 assays with controls.

Intended Use and Indications

Intended-Use: The Urine NTP Kit is designed to measure levels of neural thread protein in urine specimens from patients presenting with cognitive complaints or other signs and symptoms of suspected Alzheimer's disease (AD). Results from the Urine NTP Kit are intended for use, in conjunction with and not in lieu of current standard diagnostic procedures, to aid the physician in the diagnosis of Definite Non-AD versus Probable AD, Possible AD, or MCI.

Indications for Use: Urine NTP measurement can be used as part of diagnostic risk assessment for presence or absence of Definite Non-AD. Urine NTP has 91% specificity and is indicated for use as an adjunctive aid to help clinicians rule out definite non-AD as part of the overall diagnostic categorization, that is, only 9% of cases of definite non-AD have elevated urine NTP levels. Urine NTP has 60% sensitivity for the diagnoses of probable AD, possible AD, and MCI (as per NINCDS-ADRDA criteria, and criteria of the Quality Standards Subcommittee of the American Academy of Neurology); hence an elevated urine NTP level may help the clinician's decision for the need of further diagnostic workup (such as specialist consultations, imaging, in-depth neuropsychological testing, EEG and other testing procedures) to gain further diagnostic clarity.

Specimen Requirements

A first morning urine sample of 50 mL in a clean urine collection cup is required. The urine specimen must not be frozen before processing. Urine samples not processed immediately should be refrigerated at 2°-8° C for up to 48 hours before processing and a Stabilur tablet added to the sample. For a valid Urine NTP test, the urine sample must not be contaminated and must meet the acceptance/exclusion criteria: see 7. Specimen Requirements and Acceptance/Exclusion Criteria below.

1. Contents, Storage and Safety

#	Component	Expiry Date	Storage	Safety (See Precautions Section)
1	Microcon YM-10 Centrifugal Filter Device, (1 bag containing 11 filters), Part No. 250020			
1	Microcon YM-100 Centrifugal Filter Device, (1 bag containing 11 filters), Part No. 250021			
1	Coated microtiter plate (1 foil pouch containing 4 strips in holder), Part No. 420000		Refrigerate 2-8°C.	
1	Trisma buffered saline pH 7.0 solution, 25 mL, Nalgene bottle, Part No. 320006		Refrigerate 2-8°C.	
1	Wash Buffer (TBS with 0.05% Tween-20), 25 mL, Nalgene bottle, Part No. 320008		Refrigerate 2-8°C.	
1	Processing Buffer (10X TBS with 0.5% sodium azide), 50 mL, Nalgene bottle, Part No. 320001		Refrigerate 2-8°C.	Toxic if swallowed Hazardous waste
1	Paranitrophenol phosphate (pNPP) solution, 6 mL, Nalgene bottle, Part No. 250003		Refrigerate 2-8°C.	Irritant
1	Bovine serum albumin (BSA) powder, 10 mg, borosilicate vial, Part No. 210014		Refrigerate 2-8°C.	
1	Standard Diluent, 10 mL, Nalgene bottle, Part No. 340007		Refrigerate 2-8°C.	
1	Ammonium hydroxide 5% solution, 100 µL, borosilicate vial, Part No. 340008		Refrigerate 2-8°C.	Severe poison and extreme abrasive
1	NTP Standard powder, 1 mg, borosilicate vial, Part No. 340009		Refrigerate 2-8°C.	
1	AP Conjugate Diluent (TBS w/ 0.05% Tween 20), 10 mL, Nalgene bottle, Part No. 320008		Refrigerate 2-8°C.	

#	Component	Expiry Date	Storage	Safety (See Precautions Section)
1	Alkaline phosphatase conjugate in TBS (frozen), 200 µL, borosilicate vial, Part No. 340004		Keep frozen ≤ -20° C	
1	High NTP human urine control (frozen), 600 µL, borosilicate vial, Part No. 340001		Keep frozen ≤ -20° C	Human source material
1	Medium NTP human urine control (frozen), 600 µL, borosilicate vial, Part No. 340002		Keep frozen ≤ -20° C	Human source material
1	Low NTP human urine control (frozen), 600 µL, borosilicate vial, Part No. 340003		Keep frozen ≤ -20° C	Human source material
1	Microcon Microcentrifuge tube for use with Microcon Filters (1 bag containing 23 tubes), Part No. 250056			
1	Balancing unit (Microcon Microcentrifuge tube with Microcon Filter attached) (1 bag containing 1 tube and filter), Part No. 250057			

2. Precautions

- 2.1. Human source material used in the preparation of control reagents. Because no known test method can offer complete assurance that infectious agents are absent, handle control reagents and patient samples as if capable of transmitting infectious disease (HHS Publication No. (CDC) 93-8395. 1993).
- 2.2. Paranitrophenol phosphate (pNPP) solution: irritant; in case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- 2.3. Sodium azide in Processing Buffer, (0.5%): toxic if swallowed; after contact with skin wash immediately with plenty of soapy water.
- 2.4. Ammonium hydroxide 5% solution (used in Standard Solution preparation) (100 µL): severe poison and extreme abrasive; in case of contact with skin or eyes, immediately flush with plenty of water for at least 15 minutes. If there is eye contact, medical advice should then be sought.

3. Receiving and Storage

- 3.1 On receipt, confirm that:
 - Packaging and containers are intact;
 - Components are not past their expiry dates; and
 - Components listed as “frozen” above in Section 1 show no signs of thawing.
- 3.2 Store components requiring refrigeration or freezing as soon as possible.

4. Material and Equipment Required But Not Included

- 4.1. Plate reader and software
- 4.2. Microfuge capable of 10,000 RPM
- 4.3. Laboratory balance capable of measuring 0.5 g.
- 4.4. 50 mL Centrifuge tube and clinical centrifuge
- 4.5. Coming 50 mL 0.22 µm acetate filter unit.
- 4.6. 5 mL tube (for creatinine testing of urine specimen)
- 4.7. Pipettes:
 - 4.7.1. Multichannel Pipette 50-200 µL
 - 4.7.2. Pipette 200-1000 µL
 - 4.7.3. Pipette 1-10 µL
 - 4.7.4. Pipette 1-200 µL
- 4.8. Refrigerated storage facilities (2°– 8°C.)
- 4.9. Freezer storage facilities (≤ -20°C.)
- 4.10. Urinalysis Test Strips

5. Neural Thread Protein

The NTP 375 amino acid predicted sequence was derived from cDNA extracted from postmortem AD brain (1). Monoclonal specific anti-NTP antibodies raised against cloned recombinant NTP selectively stain degenerating neurons in AD brain, in relation to neuritic plaques and neurofibrillary tangles (2,3). NTP *in vitro* leads to neuronal cell sprouting and premature cell death (1-5). In transgenic animal studies, NTP leads to behavioral abnormalities and reproduces many of the histological changes found in AD brain (cell death, amyloid formation, phospho-tau production). NTP has pathophysiological significance and is clearly associated with the underlying disease processes (structural hallmarks of plaques and tangles) in AD brain (1-8).

6. Intended Use and Indications

Intended-Use: The Urine NTP Kit is designed to measure levels of neural thread protein in urine specimens from patients presenting with cognitive complaints or other signs and symptoms of suspected Alzheimer's disease (AD). Results from the Urine NTP Kit are intended for use, in conjunction with and not in lieu of current standard diagnostic procedures, to aid the physician in the diagnosis of Definite Non-AD versus Probable AD, Possible AD, or MCI.

Indications for Use: Urine NTP measurement can be used as part of diagnostic risk assessment for presence or absence of Definite Non-AD. Urine NTP has 91% specificity and is indicated for use as an adjunctive aid to help clinicians rule out definite non-AD as part of the overall diagnostic categorization; that is, only 9% of cases of definite non-AD have elevated urine NTP levels. Urine NTP has 60% sensitivity for the diagnoses of probable AD, possible AD, and MCI (as per NINCDS-ADRDA criteria, and criteria of the Quality Standards Subcommittee of the American Academy of Neurology); hence an elevated urine NTP level may help the clinician's decision for the need of further diagnostic workup (such as specialist consultations, imaging, in-depth neuropsychological testing, EEG and other testing procedures) to gain further diagnostic clarity."

7. Specimen Requirements and Acceptance/Exclusion Criteria

7.1 A first morning urine sample of 30 to 50 mL is required. The urine specimen must not be frozen before processing.

7.2 Acceptance/Exclusion Criteria: For a valid Urine NTP Test, the urine sample must meet the following acceptance/exclusion criteria:

7.2.1 A normal urinalysis. Urine samples with abnormal urinalysis results (such as high protein, leukocytes, red blood cells or bacterial contamination) must be excluded.

7.2.2 A urine creatinine concentration between 50 mg/dL and 225 mg/dL. Urine samples with a creatinine below 50 mg/dL (indicating that the sample is likely not a first morning void) must be excluded. Samples with creatinine above 225 mg/dL are associated with nonspecific excessive solute concentration which can lead to false positive signal and must be excluded.

7.2.3 The urine sample must not have been frozen before processing.

If the urine is unacceptable as per any of 7.2.1, 7.2.2 or 7.2.3 above, obtain a new first morning urine sample from the subject.

The above abnormalities of urine sample, sample handling, or non-first morning urine interfere with and/or invalidate the assay. Infection, protein in urine or urinary creatinine concentration >225 mg/dL are associated with false positive signals. Urinary creatinine concentration <50 mg/dL may cause false negatives. Urine that has been frozen before processing is associated with errors due to analyte aggregation and degradation.

8. Method

A. Principle of the Procedure

The assay is a competitive affinity assay that detects NTP. NTP in patient urine sample, control 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, binding is decreased in proportion to the amount of NTP present. The sample concentration of NTP is read off the curve generated by the standards.

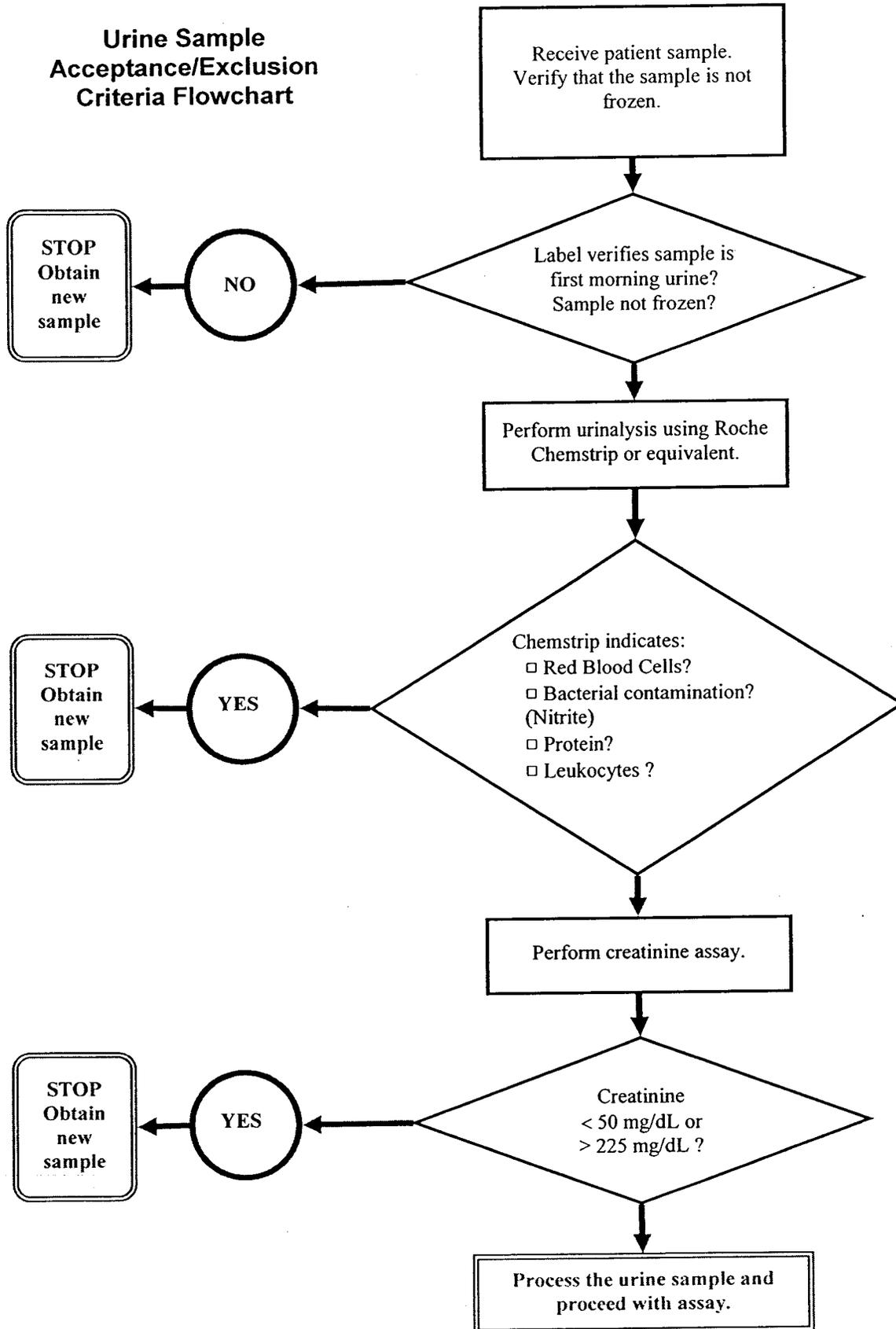
B. Quality Control

Controls should be run on each plate. Urine controls cover three ranges of analyte, namely a high control, a medium control, and a low control. Two of the three controls must be acceptable for the assay run to be acceptable.

C. Specimen Collection

1. Obtain a first morning void. Collect 30 to 50 mL of urine in a clean urine collection cup.
2. Label patient sample. The label must conform to the individual institutional guidelines, and should include verification that the sample is a first morning urine void. **A non-first morning sample invalidates the assay.**
3. Urine can be processed immediately or refrigerated at 2-8° C up to 48 hours before processing, provided it is not contaminated or otherwise excluded (see 7.2 Acceptance/Exclusion Criteria above).
4. Specimen transport: If the urine sample is to be transported it should be kept refrigerated (2-8° C) at all times, and a Stabilur tablet should be added to the sample. **The NTP test is not valid on frozen urine.** Urine can be refrigerated up to 48 hours before processing provided it is not contaminated or otherwise excluded.

Urine Sample Acceptance/Exclusion Criteria Flowchart



D. Specimen Processing

1. Urine samples are received from the specimen receiving and handling area. Record unique patient ID# on all urines received. The label must conform to the individual institutional guidelines, and should include verification that the sample is a first morning urine void. **A non-first morning sample invalidates the assay.**
2. Perform urinalysis using Roche Chemstrip or equivalent method. Do not use any samples that do not meet the specifications detailed under 7.2 Acceptance/Exclusion Criteria, above. If the urine is unacceptable, notify provider and dispose of urine accordingly. A new first morning urine sample should be obtained from the subject.
3. Record urine sample ID # on a 5 mL tube (not provided) and a 50 mL tube (not provided) for each urine. Pour at least 4 mL of corresponding urine into 5 mL tube for creatinine testing and up to 45 mL urine into the 50 mL tube. Repeat for each urine.
4. Assay creatinine value. Do not use any samples that have a creatinine value above 225 mg/dL or below 50 mg/dL: see 7.2 Acceptance/Exclusion Criteria, above. If the urine is unacceptable, notify provider and dispose of urine accordingly. A new first morning urine sample should be obtained from the subject.
5. Centrifuge urine in the 50 mL tube at 3000 x g for 15 minutes to remove cellular debris.
6. Filter urine through a 0.22 µm cellulose acetate filter (Corning) using a 50 mL collection tube. Verify that the sample ID# and date of receipt is recorded on the side of the 50 mL tube. Note: this filter is not provided in Urine NTP Test kit: see 4. Material and Equipment Required But Not Included above.
7. Add 1:10 volume Processing Buffer (0.5% sodium azide in 10X TBS) to the filtrate in order to bring the filtrate to 0.05% Azide in TBS. Label with ID # and "F+P" (Filtered and Preserved). Proceed to step 8. directly or store at $\leq -20^{\circ}\text{C}$ until proceeding to step 8.
8. Pipette 0.5 mL of F+P labeled urine (or urine control) into the top of Microcon® YM-10 filter unit.
9. Spin in a microcentrifuge at 10,000 RPM for 30 minutes.
10. Remove Microcon® YM-10 from centrifuge. Discard the pass through (liquid in bottom of unit).
11. Reconstitute the retentate (material above filter) to approximately 0.5 mL with 1X TBS.
12. Using the same Microcon® YM-10, centrifuge at 10,000 RPM for 15 minutes.
13. Remove Microcon® YM-10 from centrifuge. Discard the pass through.
14. Reconstitute the retentate to 0.5 mL with 1x TBS.
15. Using the same Microcon® YM-10, centrifuge at 10,000 RPM for 15 minutes.
16. Remove Microcon® from centrifuge. Discard the pass through.
17. On a balance weigh and tare a YM-100 filter unit (Blue). Add the retentate to top of unit and add sufficient TBS to bring mass to $0.5\text{ g} \pm 0.01\text{ g}$.
18. Spin Microcon® YM-100 at 10,000 RPM for 5 minutes. Keep the pass through (liquid in bottom of unit).
19. The sample (the pass through) can be tested immediately or stored frozen at -20°C for up to one year before testing. Do not freeze and thaw processed samples more than twice. Do not use urine that has been frozen before processing (see 7.2.3 above, Acceptance/ Exclusion Criteria).

E. Protocol for the Urine NTP Assay using Processed Urine

1. Add 6 µL of ammonium hydroxide to Standard Diluent.
2. Add 1mL of Standard Diluent with ammonium hydroxide to standard powder and let stand for at least 1 hour.
 - a. Dilute Standard to 80 µg/mL by adding 0.25 mL to 2.875 mL of 1X TBS
 - b. Dilute 1 mL 80 µg/mL Standard with 1 mL of 1X TBS to make 40 µg/mL Standard.
 - c. Repeat dilution 1 mL of 40 µg/mL to 1 mL TBS to make 20 µg/mL and repeat dilution 20 µg/mL to make 10 µg/mL Standards.
 - d. Use TBS only for 0 µg/mL Standard.
3. Thaw Controls. Bring to room temperature for 30 minutes.
4. Remove the microtiter plate, the samples, and all buffers from the refrigerator.
5. Add BSA to AP Conjugate Diluent.
6. Add 200 µL of AP Conjugate to 9.8 mL of AP Conjugate Diluent with BSA.
7. Add 50 µL of Standard, Control or sample to the wells; then add 50 µL of AP Conjugate to wells of the plate.
8. Incubate 1 hour at room temperature.
9. Dump plate. Bang on paper towels and wash with Wash Buffer using a multi-channel pipette (200 µL per well). Repeat 2X for a total of 3 washes.
10. Add 150 µL of pNPP and allow the assay plate to develop.
11. Check the optical density at 405 nm after 10 minutes and, if necessary, thereafter every three to five minutes until the Optical Density of the 0 µg/mL (TBS) Standard is between 2.0 and 2.1.
12. Read concentration results off the semi-log curve.

F. Results

1. Read the results of the patient sample, Urine NTP Controls, and the Urine NTP Standards on the plate reader using a semi-log curve. Test results are calculated in concentration of NTP in $\mu\text{g/mL}$ from a standard curve generated by a semi-log fit to the absorbance values of the respective Standards.
2. Acceptance criteria: both 0 $\mu\text{g/mL}$ (TBS) Standards must develop to at least 2.0 O.D. at 405 nm within 120 minutes after the addition of pNPP to the plate: see E.11 above.
3. For each of the High, Medium and Low-NTP Controls, determine the two NTP $\mu\text{g/mL}$ values and calculate the mean of the two results.

Acceptance criteria: Acceptable if two out of the three NTP Control mean values are within range, that is:

1. High-NTP Control mean value is between 47- 71 $\mu\text{g/mL}$; AND/OR
2. Medium-NTP Control mean value is between 19- 35 $\mu\text{g/mL}$; AND/OR
- 3.
4. Low-NTP Control mean value is between 11- 18 $\mu\text{g/mL}$.

Urine Controls	Acceptance Criteria for Mean Value	Results	Mean Value of Results	Acceptable	
				YES	NO
High-NTP Control	47-71 $\mu\text{g/mL}$				
Medium-NTP Control	19 – 35 $\mu\text{g/mL}$				
Low-NTP Control	11 – 18 $\mu\text{g/mL}$				
Urine NTP Controls	2 out of 3 NTP Controls meet Acceptance Criteria				

4. For each of the Standards (80 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 20 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$ and 0 $\mu\text{g/mL}$ (TBS) Standards), determine the two OD values (405 nm) and calculate the mean value of the two results.

Acceptance criteria: Acceptable if:

1. 80 $\mu\text{g/mL}$ Standard mean OD value is between 0.171 to 0.425;
2. 40 $\mu\text{g/mL}$ Standard mean OD value is between 0.393 to 0.661; AND
2. 20 $\mu\text{g/mL}$ Standard mean OD value is between 0.798 to 1.120; AND
4. 10 $\mu\text{g/mL}$ Standard mean OD value is between 1.041 to 1.483; AND
5. 0 $\mu\text{g/mL}$ (TBS) Standard mean OD values is between 2.0 to 2.1.

Urine NTP Standard	Acceptance Criteria for Mean OD Value	Individual OD Results	Mean OD Value of Results	Acceptable	
				YES	NO
80 µg/mL	0.171 to 0.425				
40 µg/mL	0.393 to 0.661				
20 µg/mL	0.798 to 1.120				
10 µg/mL	1.041 to 1.483				
0 µg/mL (TBS)	2.0 to 2.1				
Urine NTP Test Standards	The mean OD values of all 5 Urine NTP Controls meet Acceptance Criteria.				

5. Calculate the R value and R² value for the curve fit for the semi-log curve. Acceptance criteria:

Acceptable if:

1. $R \geq .9$ AND
2. $R^2 \geq .81$

6. Report the patient NTP results if all Acceptance Criteria met. Otherwise, repeat assay.

9. Interpretation

The urine NTP assay measures the concentration of neural thread protein in first morning urine. The test results are invalid for urine under any of the following circumstances, where a new first morning urine sample should be provided: 1) non-first morning sample, 2) frozen urine, 3) urinary tract infection or contaminated urine, 4) glycosuria 5) proteinuria, 6) presence of nitrites, or 7) urine creatinine concentration <50 mg/dL or >225 mg/dL. Normal reference range for urine NTP is ≤ 22 µg/mL (<10% of normal non-demented individuals were found to have urine NTP test values >22 µg/mL). In a multicenter blinded prospective trial involving 200 patients with cognitive symptoms who were tested with the urine NTP test and then evaluated by specialists for periods up to over one year, at a cutoff value of 22 µg/mL, an elevated urine NTP test agreed 89% with the eventual diagnosis of probable Alzheimer's disease. A urine NTP test value ≤ 22 µg/mL agreed 91% with the eventual diagnosis of definite non-Alzheimer's disease.

Intended-Use: The Urine NTP Kit is designed to measure levels of neural thread protein in urine specimens from patients presenting with cognitive complaints or other signs and symptoms of suspected Alzheimer's disease (AD). Results from the Urine NTP Kit are intended for use, in conjunction with and not in lieu of current standard diagnostic procedures, to aid the physician in the diagnosis of Definite Non-AD versus Probable AD, Possible AD, or MCI.

Indications for Use: Urine NTP measurement can be used as part of diagnostic risk assessment for presence or absence of Definite Non-AD. Urine NTP has 91% specificity and is indicated for use as an adjunctive aid to help clinicians rule out definite non-AD as part of the overall diagnostic categorization; that is, only 9% of cases of definite non-AD have elevated urine NTP levels. Urine NTP has 60% sensitivity for the diagnoses of probable AD, possible AD, and MCI (as per NINCDS-ADRDA criteria, and criteria of the Quality Standards Subcommittee of the American Academy of Neurology); hence an elevated urine NTP level may help the clinician's decision for the need of further diagnostic workup (such as specialist consultations, imaging, in-depth neuropsychological testing, EEG and other testing procedures) to gain further diagnostic clarity."

10. Non-Clinical Studies of the Urine NTP Assay

1. Drug Interference Studies: Low, medium, and high NTP urines were tested before and after spiking with the following drugs at 25 µg/mL concentration acetaminophen, alprazolam, cephalexin, diltiazem, furosemide, capoten, potassium, fosamax, lanoxin, lipitor, losec, pepcid, vasotec, zoloft, adalat, atenolol, glyburide, hydrochlorothiazide, metoprolol, temazepam, amoxicillin, ativan, b iaxin, d emerol, i ndur, norvasc, tetracycline, synthroid, prozac, flurazepam, i buprofen, c oumadin, metformin, codeine phosphate. None of the spiked drugs had significant effect on the NTP urine test values of any of the urine samples.
2. 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 (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.
3. 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.
4. 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%.
5. Analytical Threshold: The limit of detection (at 405 nm) is OD 1.315 ± 0.8, corresponding to a threshold of 10 µg/mL.
6. 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).
7. Stability Studies: Stability studies indicate that refrigerated coated plates are stable for 8 weeks. Standard is stable for ≥ 6 months. Other kit components have stability ≥ 1 year. (see 1. Contents above). Urine NTP test kits must not be used after the kit label expiry date.
8. 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. High, medium, and low-NTP processed urine samples were assayed, frozen at -80°C, and then tested in 135 replicates on 45 different days up to 67 days. The CVs of the individual samples were 5.6% (high-NTP urine); 11.8% (medium-NTP urine); and 9.8% (low-NTP urine).
9. Normal reference range study: 122 normal individuals (with no history of cognitive complaints, symptoms, or disorders) were assayed. 90% of reference individuals had urine NTP ≤ 22 µg/mL. There were no differences according to age or sex.

11. References

1. de la Monte SM, et al. Characterization of the AD7CTM-NTP cDNA expression in Alzheimer's disease and measurement of a 41-kD protein in cerebrospinal fluid. *J Clin Invest* 1997; 100: 3093-3104.
2. de la Monte SM, Carlson RI, et al. Profiles of neuronal thread protein expression in Alzheimer's disease. *J Neuropathol Exp Neurol* 1996; 55: 1038-1050.
3. de la Monte SM, Xu YY, et al. Modulation of neuronal thread protein expression with neuritic sprouting: relevance to Alzheimer's disease. *J Neurol Sci* 1996; 138: 26-35.
4. de la Monte SM, Wands JR. The AD7CTM-NTP neuronal thread protein biomarker for detecting Alzheimer's disease. *J Alz Dis* 2001; 3: 345-353.
5. de la Monte SM, Wands JR. Alzheimer-associated neuronal thread protein-induced apoptosis and impaired mitochondrial function in human central nervous system-derived neuronal cells. *J Neuropathol Exp Neurol*, 2001; 60 (2): 195-207.
6. Munzar M, Levy S, et al. Competitive ELISA format urinary assay of neural thread protein in Alzheimer's disease. *Alzheimer's Reports* 2001; 4 (2): 61-65.
7. Munzar M, Levy S, et al. Clinical study of a urinary competitive ELISA for neural thread protein in Alzheimer's disease. *Neurol Clin Neurophysiol* 2002; 1: 1-7.
8. Munzar M, McConville M, et al. A retrospective clinical study of urinary neural thread protein in Alzheimer's disease. *Alzheimer's Reports*. 2002; 5(1): 1-6.