Neurodegenerative Diseases (AD, PD, HD) Diagnostics

1. Introduction

What are Neurodegenerative Diseases?

Neurodegenerative diseases are defined as diseases described by the progressive dysfunction or death of neurons or nerve cells in the human body. These diseases mainly target the central nervous system meaning the function of the affected body gradually diminishes and reaches a state of severe disability or the patient dies. Some of the familiar brain disorders associated with neurodegenerative diseases are Parkinson's Disease, Alzheimer's Disease and Huntington's Disease [1-3].

The main characteristics of these diseases are:

- **Progressive Deterioration:** Symptoms and functions worsen over time.
- **Irreversibility:** Currently, most neurodegenerative diseases have no cure, and treatments can only alleviate symptoms and slow disease progression.
- **Multifactorial Etiology:** These diseases are influenced by both genetic and environmental as well as lifestyle factors.

Why is early diagnosis important?

For several reasons, neurodegenerative disease requires early diagnosis and effective treatment.

- **Improving Quality of Life:** Early detection and intervention can slow disease progression, alleviate symptoms, and enhance the quality of life for patients.
- **Prolonging Life:** Early treatment can extend the life expectancy of patients by delaying the severe outcomes associated with these diseases.
- **Reducing Societal Burden:** Early diagnosis and treatment can lower healthcare costs, reduce the economic and psychological burden on patients and their families, and lessen the overall societal impact.
- **Promoting Scientific Research:** More cases and data with early diagnosis means more to study disease mechanisms, and hopefully learn how to develop new therapies.

2. Common Degenerative Diseases

Neurodegenerative diseases are a group of disorders characterized by the gradual degeneration or death of neurons, the fundamental units of the nervous system. These conditions primarily affect the central nervous system, leading to a progressive loss of function, which can ultimately result in severe disability or death. Common neurodegenerative diseases include Parkinson's disease, Alzheimer's disease, and Huntington's disease.

Alzheimer's Disease (AD)

The most common form of dementia, Alzheimer's disease is a neurodegenerative disorder largely defined by the memory loss and subsequent drop in cognitive ability. The disease is almost always of the elderly, predominantly over the age of 65. But some people get early onset Alzheimer's disease, and start to exhibit symptoms earlier than that [4,5].

Pathological Changes

The neuropathological hallmarks of Alzheimer's disease primarily include:

- **Amyloid Plaques:** These are aggregates formed from protein fragments due to the abnormal cleavage of amyloid precursor protein.
- **Neurofibrillary Tangles:** These tangles occur because abnormal tau protein builds up in the cytoplasm of neurons, and disrupts the intracellular transport system.

Diagnosis

1. Clinical Symptom Assessment
2. Neuropsychological Testing
3. Brain Imaging Techniques
4. Biomarker Testing (Aβ42, p-tau217, p-tau181, p-tau231, GFAP, NfL)

Parkinson's Disease (PD)

Parkinson's disease is a neurodegenerative disorder that primarily affects the motor system. Such a loss of dopamine producing neurons in the substantia nigra region of the brain causes the disease, which results in less dopamine. Dopamine is as important a neurotransmitter as it is to the movement as it is to the emotional responses. These neurons are damaged or killed leading to the characteristic motor and non-motor symptoms seen in Parkinson's disease [6,7].

Pathological Changes

1. Loss of Dopamine Neurons
2. Formation of Lewy Bodies
3. Neurotransmitter Changes
4. Neurotransmitter Changes

Diagnosis

1. Clinical Symptom Assessment
2. Neurological Examination
3. Drug Response Test
4. Imaging Studies
5. Biomarker Testing (Alpha-synuclein, DJ-1, NfL)

Huntington's Disease (HD)

A CAG repeats expansion mutation of the HTT gene leading to the abnormal accumulation of the mutant huntingtin protein (mHTT) is a hereditary rare neurodegenerative disorder [8,9].

Pathological Changes

1. Neuronal Loss
2. Accumulation of Mutant Huntingtin Protein
3. Formation of Neuronal Inclusions
4. Impairment of Neural Transmission
5. Brain Atrophy

Diagnosis

1. Neurological Examination
2. Genetic Testing
3. Neuropsychological Assessment
4. Imaging Studies
5. Biomarker Testing

Table 1: Biomarkers of Neurodegenerative Diseases

Biomarker name	Alzheimer's Disease (AD)	Parkinson's Disease (PD)	Huntington's Disease (HD)
Phospho-tau181 (p-tau181)	√		
Phospho-tau217 (p-tau217)	√		
Phospho-tau231(p-tau231)	√		
Phospho-tau212 and 214 (p-tau212 and 214)	√		
Phospho-tau202 and 205 (p-tau202 and 205)	√		
Phospho-tau413 (p-tau413)	√		
Phospho-tau422 (p-tau422)	√		
Tau proteins (Tau)	√	√	
Neurofilament light chain (NfL)	√	√	√
Glial fibrillary acid protein (GFAP)	√	√	
Beta-amyloid 42 (Aβ42)	√	√	
Beta-amyloid 38 (Aβ38)	√		
Beta-amyloid 40 (Aβ40)	√	√	
Monocyte chemoattractant protein-1 (MCP-1/CCL2)	√		√
Neurogranin protein (Neurogranin)	√		
Neuron-specific enolase (NSE)	√		
sTREM2	√		
Visinin-like protein 1 (VLP-1)	√		
Chitinase-3-like protein 1 (CHI3L1/YKL-40)	√		
Alpha-synuclein (α-synuclein)	√	√	

Apolipoprotein E (APOE4)	√		
AD-associated thread protein (AD7c-NTP)	√		
Human Parkinsonism associated deglycase (PARK7)		√	√
Brain-derived neurotrophic factor (BDNF)			√
Interleukin-6 (IL-6)			√
Pro-neuropeptide Y			√
Heart fatty acid-binding protein (HFABP)	√		

Biomarkers for Alzheimer's Disease

Table 2: Key biomarkers in blood and their detection limits [10-16].

	Biomarker	Limit (Pathological concentration in blood)
Alzheimer Disease (AD)	Phospho-tau181 (p-tau181)	>3 pg/ml
	Phospho-tau217 (p-tau217)	>10 pg/ml
	Phospho-tau231(p-tau231)	>10 pg/ml
	Phospho-tau212 and 214 (p-tau212 and 214)	/
	Phospho-tau202 and 205 (p-tau202 and 205)	/
	Phospho-tau413 (p-tau413)	/
	Phospho-tau422 (p-tau422)	/
	Tau proteins (Tau)	>100 pg/ml
	Neurofilament light chain (NfL)	>30 pg/ml
	Glial fibrillary acid protein (GFAP)	>100 pg/ml
	Beta-amyloid 42 (Aβ42)	/
	Beta-amyloid 38 (Aβ38)	/
	Beta-amyloid 40 (Aβ40)	/
	Monocyte chemoattractant protein-1 (MCP-1/CCL2)	/
	Neurogranin protein (Neurogranin)	/
	Neuron-specific enolase (NSE)	/
	sTREM2	/
	Visinin-like protein 1 (VLP-1)	/

	Chitinase-3-like protein 1 (CHI3L1/YKL-40)	**/**
	Alpha-synuclein (α-synuclein)	**/**
	Apolipoprotein E (APOE4)	**/**
	AD-associated neuaronal thread protein (AD7c-NTP)	**/**
	Heart fatty acid-binding protein (HFABP)	**/**

Sample Types: Blood, cerebrospinal fluid (CSF)

Biomarkers for Parkinson's Disease

Table 3: Key biomarkers in blood and their detection limits.

	Biomarker	**Limit (Pathological concentration in blood)**
Parkinsonism Disease (PD)	**Human Parkinsonism associated deglycase (PARK7)**	**/**
	Alpha-synuclein (α-synuclein)	**>10 pg/ml**
	Tau proteins (Tau)	**>10 pg/ml**
	Neurofilament light chain (NfL)	**/**
	Glial fibrillary acid protein (GFAP)	**/**
	Beta-amyloid 42 (Aβ42)	**/**
	Beta-amyloid 40 (Aβ40)	**>100 pg/ml**

Sample Types: Blood and cerebrospinal fluid (CSF)

Biomarkers for Huntington's Disease

Table 4: Key biomarkers in blood and their detection limits.

	Biomarker	Limit (Pathological concentration in blood)
Huntington's Disease (HD) Biomarker	Brain-derived neurotrophic factor (BDNF)	/
	Monocyte chemoattractant protein-1 (MCP-1/CCL2)	/
	Interleukin-6 (IL-6)	/
	Neurofilament light chain (NfL)	/
	Pro-neuropeptide Y	/

Sample Types: Blood, cerebrospinal fluid (CSF)

3. Technologies and Platforms for Neurodegenerative Diseases Diagnostics

ELISA (Enzyme-Linked Immunosorbent Assay)

- **Overview:** It is a commonly used detection technology that determines the presence or absence of specific proteins or other molecules contained in a sample and combines antibodies and an enzyme label.
- **Advantages:** High sensitivity, strong specificity, simple operation, and relatively low cost.

Simoa (Single Molecule Array)

- **Overview:** Unlike traditional ELISA techniques, this technology can detect very low levels of proteins, nucleic acids and other biomolecules at a sensitivity several orders of magnitudes greater. Through using an array of sealed microwells and fluorescently labeled single molecules, Simoa is able to achieve detection and quantification of individual molecules. This capability is essential for early disease diagnosis, biomarker detection, scientific research [17-19].
- **Advantages:**

  (1) Ultra-high Sensitivity: Simoa can detect biomarkers at extremely low concentrations, even at the

single-molecule level, which is several orders of magnitude more sensitive than traditional methods such as ELISA.

(2) Early Disease Detection: Due to its high sensitivity, Simoa technology can detect trace biomarkers at very early stages of diseases, facilitating early diagnosis and treatment.

(3) Accurate Quantification: Simoa not only detects the presence of biomarkers but also accurately quantifies their concentrations, which is crucial for precise disease monitoring and treatment.

(4) Versatility and Flexibility: The application of this technology extends to other biomolecules, such as proteins and nucleic acids, thus it is extremely valuable for a wide range of biomedical research and clinical applications.

(5) Reduced Sample Volume: Simoa requires only a very small amount of sample for testing, reducing the need for large sample volumes from patients.

(6) High Throughput and Automation: The Simoa platform supports high-throughput sample analysis and can be automated, improving the efficiency and reproducibility of experiments.

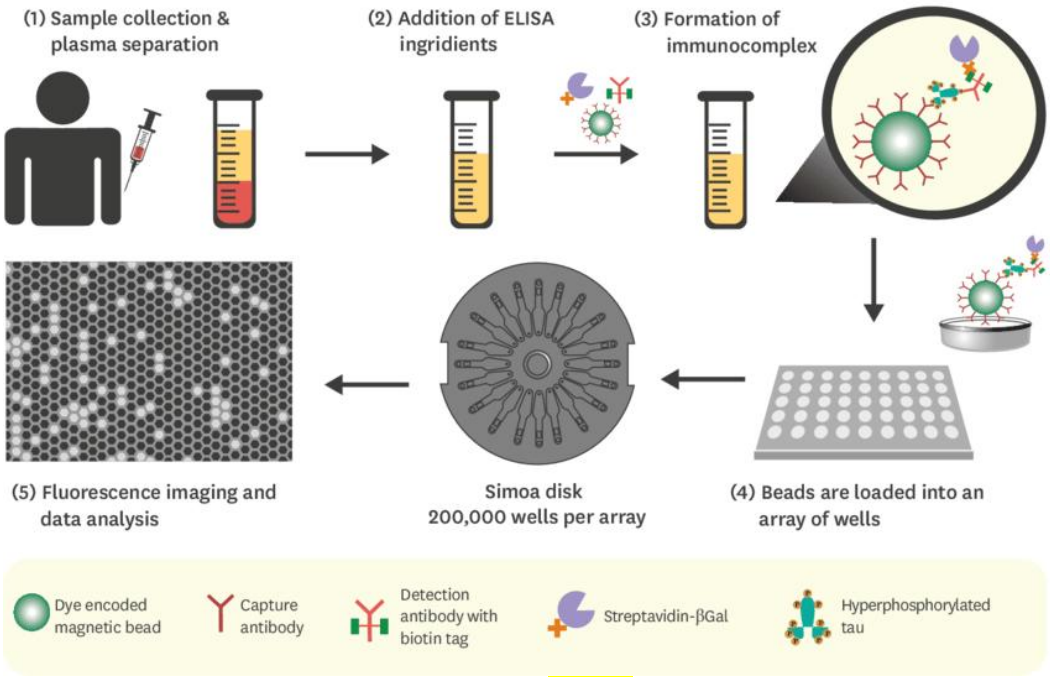


Figure: Detecting misfolded proteins in blood through SIMOA technology for the diagnosis of Alzheimer's disease / Source: researchgate.net

Multiplex Assays

- **Overview:** Simultaneous detection of multiple biomarkers with enhanced detection efficiency and increased data throughput achieved with different fluorescent or chemically labels specifying each biomarker.

- **Advantages:** High throughput, sample and reagent savings, strong data integration.

ECL (Electrochemiluminescence)

- **Overview:** A high-sensitivity detection method that combines electrochemical and luminescence technologies to analyze biomarkers and molecular interactions.
- **Advantages:** Extremely high sensitivity and specificity, low background noise, suitable for detecting low-concentration samples and high-throughput screening.

LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry)

- **Overview:** A high-precision detection method that combines liquid chromatography and mass spectrometry to analyze compounds in complex biological samples.
- **Advantages:** High sensitivity and specificity, capable of detecting multiple target molecules simultaneously, suitable for metabolomics and proteomics research.

ECLIA (Electrochemiluminescence Immunoassay)

- **Overview:** A combined electrochemical-luminescence immunoassay method for biomarker analysis.
- **Advantages:** Extremely high sensitivity and specificity, suitable for detecting low-concentration samples, low background noise.

CLEIA (Chemiluminescent Enzyme Immunoassay)

- **Overview:** A chemiluminescence enzyme linked immunoassay (CLIEA) method for detection of biomarkers through a chemical reaction that in a light signal.
- **Advantages:** High sensitivity, high specificity, rapid detection, suitable for analyzing various sample types.

4. Clinical Applications and Research in Alzheimer's Disease Diagnostics

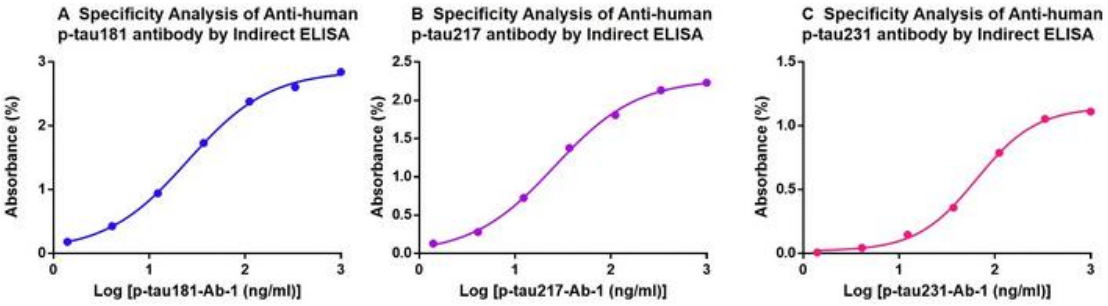
Name	Sample	Biomarkers	Method
Roche	Blood	P-tau217	ECL
	CSF	P-tau181/Aβ42 ratio	
	Blood	P-tau181+APOE E4	
C_2N Diagnostics	Blood	Aβ42/40 ratio and p-tau217 ratio	LC-MS
Quest Diagnostics	Blood	Aβ42/40	LC-MS/MS
		p-tau217	ECLIA
		p-tau181	IA

Quanterix	Blood	p-tau181	Simoa
		NfL	
		p-tau217	
Alzpath	Blood	p-tau217	Simoa
Labcorp	Blood	p-tau217	CLEIA
		Aβ42/40	
		GFAP	
		p-tau181	
		NfL	

5. GeneMedi' s Alzheimer's Disease Diagnostics Solution

	Name	Cat No.
Genemedi's Alzheimer's disease-related biomarker products	Phospho-tau181 (p-tau181)	GMP-h-p-tau181
	Phospho-tau217 (p-tau217)	GMP-h-p-tau217
	Phospho-tau231(p-tau231)	GMP-h-p-tau231
	Phospho-tau212 and 214 (p-tau212 and 214)	GMP-h-p-tau212/214
	Phospho-tau202 and 205 (p-tau202 and 205)	GMP-h-p-tau202/205
	Phospho-tau413 (p-tau413)	GMP-h-p-tau413
	Phospho-tau422 (p-tau422)	GMP-h-p-tau422
	Tau proteins (Tau)	GMP-h-Tau
	Neurofilament light chain (NfL)	GMP-h-NfL
	Glial fibrillary acid protein (GFAP)	GMP-h-GFAP
	Beta-amyloid 42 (Aβ42)	GMP-h-Aβ42
	Beta-amyloid 38 (Aβ38)	GMP-h-Aβ38
	Beta-amyloid 40 (Aβ40)	GMP-h-Aβ40
	Monocyte chemoattractant protein-1 (MCP-1/CCL2)	GMP-h-MCP-1
	Neurogranin protein (Neurogranin)	GMP-h-Neurogranin
	Neuron-specific enolase (NSE)	GMP-h-NSE
	sTREM2	GMP-h-sTREM2
	Visinin-like protein 1 (VLP-1)	GMP-h-VLP-1
	Chitinase-3-like protein 1 (CHI3L1/YKL-40)	GMP-h-YKL-40
	Alpha-synuclein (α-synuclein)	GMP-h-α-synuclein
	Apolipoprotein E (APOE4)	GMP-h-APOE4
	AD-associated neuaronal thread protein (AD7c-NTP)	GMP-h-AD7c-NTP
	Heart fatty acid-binding protein (HFABP)	GMP-h-HFABP

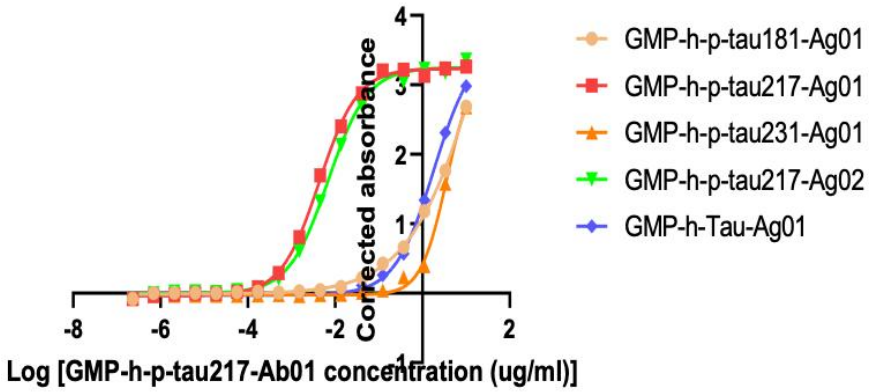
1) GeneMedi's three phosphorylated tau protein antibodies (p-tau181, p-tau217, and p-tau231) demonstrate outstanding sensitivity and exceptional specificity with their corresponding phosphorylated forms in direct ELISA assays.



Antigen	GMP-h-p-tau181-Ag01	GMP-h-p-tau217-Ag01	GMP-h-p-tau231-Ag01
Antibody	GMP-h-p-tau181-Ab01	GMP-h-p-tau217-Ab01	GMP-h-p-tau231-Ab01
EC50	25.26 ng/ml	25.85 ng/ml	64.34 ng/ml

Fig 1. Validation of GeneMedi's Anti-human p-tau181/p-tau217/p-tau231 antibody.

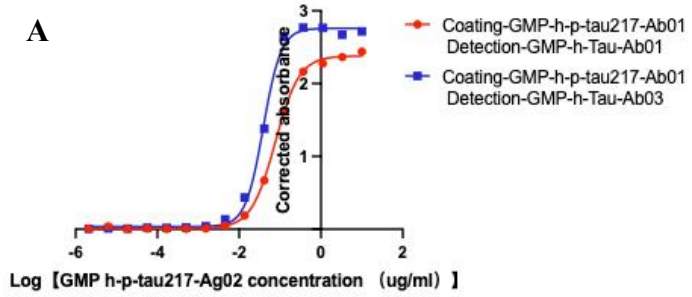
2) GeneMedi's three phosphorylated tau protein antibodies (p-tau181, p-tau217, and p-tau231) show excellent sensitivity and extreme specificity with their corresponding phosphorylated forms in direct ELISA assays.



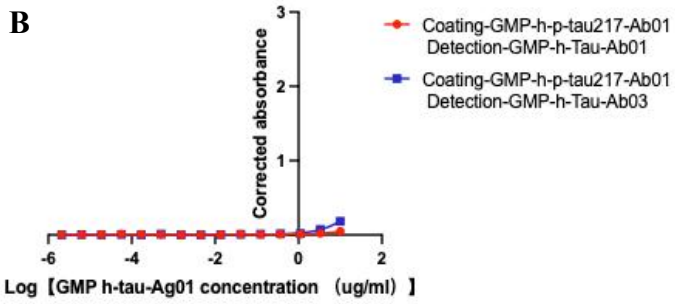
Antibody	GMP-h-p-tau217-Ab01				
Antigen	GMP-h-p-tau181-Ag01(peptide)	GMP-h-p-tau217-Ag01(peptide)	GMP-h-p-tau231-Ag01(peptide)	GMP-h-p-tau217-Ag02 (full length)	GMP-h-Tau-Ag01(full length)
EC50	27320 ng/ml	4.326 ng/ml	3563 ng/ml	6.943 ng/ml	1843 ng/ml

Fig 2. Direct ELISA Binding Curves of Phosphorylated Tau217 Antibody with Various Antigens.

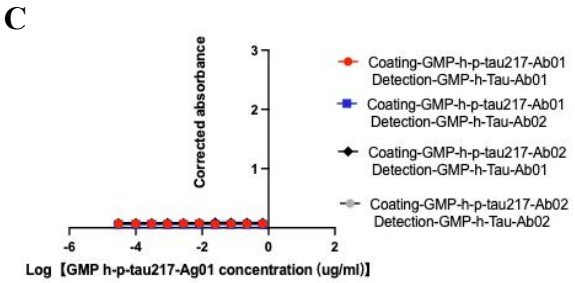
3) GeneMedi's three phosphorylated tau protein antibodies (p-tau181, p-tau217, and p-tau231) display outstanding sensitivity and exceptional specificity to their corresponding phosphorylated forms in direct ELISA assays.



Antigen	GMP-h-p-tau217-Ag02 (full length)	
Antibody pair	Coating-GMP-h-p-tau217-Ab01 Detection-GMP-h-Tau-Ab01	Coating-GMP-h-p-tau217-Ab01 Detection-GMP-h-Tau-Ab03
EC50	76.55 ng/ml	39.02 ng/ml



Antigen	GMP-h-tau-Ag01 (full length)	
Antibody pair	Coating-GMP-h-p-tau217-Ab01 Detection-GMP-h-Tau-Ab01	Coating-GMP-h-p-tau217-Ab01 Detection-GMP-h-Tau-Ab03
EC50	4352000 ng/ml	20030 ng/ml



Antigen	GMP-h-p-tau217-Ag01 (peptide)			
Antibody pair	Coating-GMP-h-p-tau 217-Ab01 Detection-GMP-h-Tau-Ab01	Coating-GMP-h-p-tau 217-Ab01 Detection-GMP-h-Tau -Ab02	Coating-GMP-h-p-tau21 7-Ab02 Detection-GMP-h-Tau-A b01	Coating-GMP-h-p-tau21 7-Ab02 Detection-GMP-h-Tau-A b02
EC50	NA	NA	NA	NA

Fig 3. Sandwich ELISA Analysis of Full-Length Phosphorylated Tau 217 Antigen with Corresponding Phosphorylated Tau 217 Antibody and Total Tau Antibody.

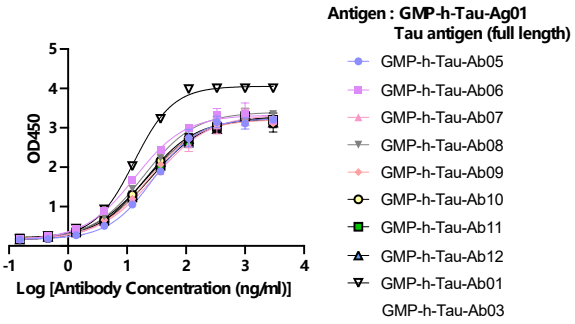
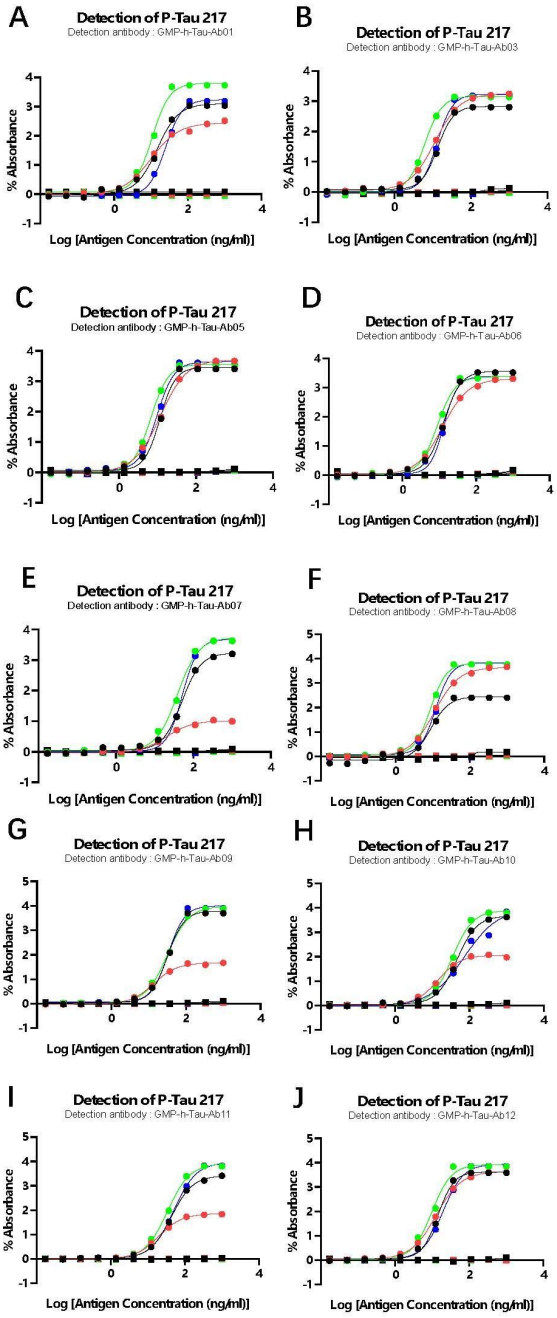
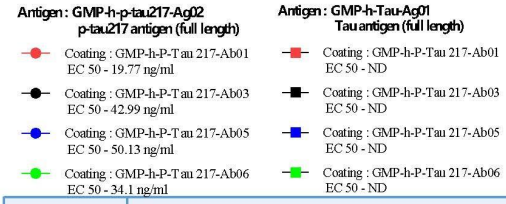


Fig4. GMP-h-Tau-Ab01/03/05/06/07/08/09/10/11/12 (Anti-human Tau antibody) is validated to detect the GMP-h-Tau-Ag01 (Recombinant human Tau Protein) in ELISA.





	Capture Antibody			
Detection Antibody	GMP-h-P-Tau 217-Ab01	GMP-h-P-Tau 217-Ab03	GMP-h-P-Tau 217-Ab05	GMP-h-P-Tau 217-Ab06
GMP-h-Tau-Ab01	10.16	14.85	20.43	10.79
GMP-h-Tau-Ab03	11.36	13.4	13.08	6.15
GMP-h-Tau-Ab05	11.90	11.63	9.60	6.70
GMP-h-Tau-Ab06	12.45	13.10	13.34	8.90
GMP-h-Tau-Ab07	19.90	50.42	51.51	31.80
GMP-h-Tau-Ab08	11.18	9.03	12.16	8.70
GMP-h-Tau-Ab09	13.81	31.73	33.16	31.93
GMP-h-Tau-Ab10	14.25	45.55	72.04	34.43
GMP-h-Tau-Ab11	19.77	42.99	50.13	34.10
GMP-h-Tau-Ab12	12.74	14.30	19.56	9.80

Fig. Sandwich ELISA Analysis of Full-Length Phosphorylated Tau 217 Antigen with Corresponding Phosphorylated Tau 217 Antibody and Total Tau Antibody.

The table shows the EC50 values of different antibody combinations in sandwich ELISA experiments targeting the GMP-h-p-tau217-Ag02 antigen (full-length), as shown in **A–J**. GMP-h-p-Tau217-Ab06 shows the best EC50 in almost all antibody pairs.

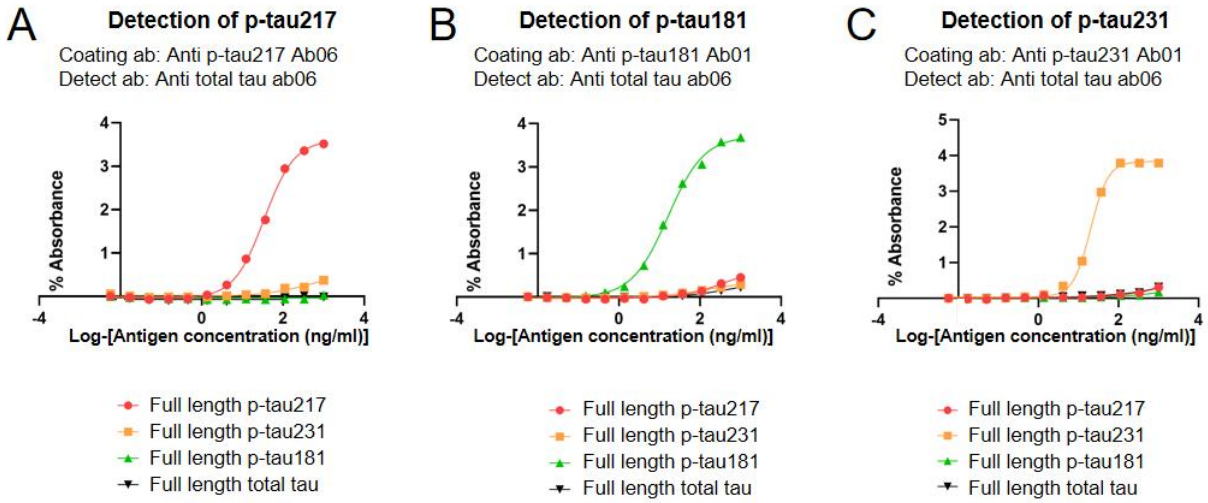


Fig6. High specificity and reliability of p-Tau217/181/231 and tau antibody pairs In ELISA.

A. p-Tau217/ tau antibody pair binds specifically to p-Tau217 full-length antigen.

B. p-Tau181/ tau antibody pair binds specifically to p-Tau181 full-length antigen.

C. p-Tau231/ tau antibody pair binds specifically to p-Tau231 full-length antigen.

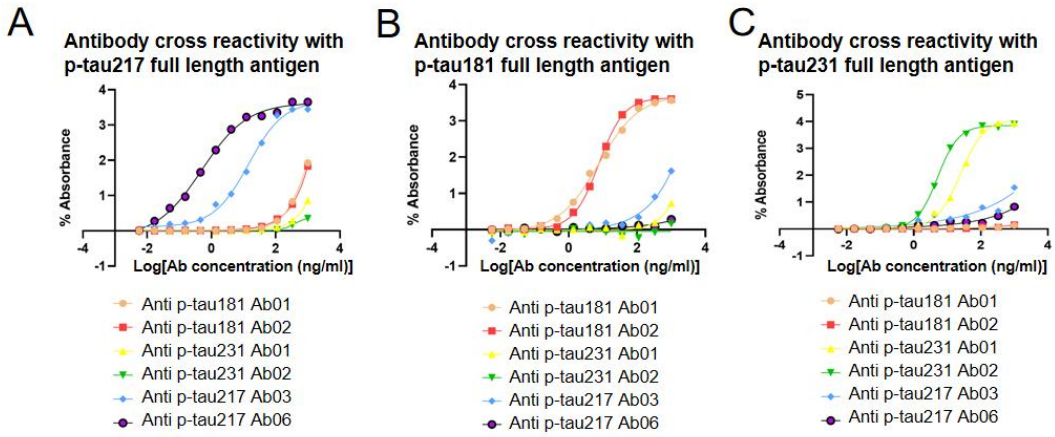


Fig7. High specificity and reliability of p-Tau217/181/231 Phosphorylated Full-Length Antigens In ELISA.

A. p-Tau217 full-length antigen binds specifically to p-Tau217 antibodies.

B. p-Tau181 full-length antigen binds specifically to p-Tau181 antibodies.

C. p-Tau231 full-length antigen binds specifically to p-Tau231 antibodies.

Reference

[1] Dugger BN, Dickson DW. Pathology of Neurodegenerative Diseases. Cold Spring Harb Perspect Biol. 2017 Jul 5;9(7):a028035.

[2] Temple S. Advancing cell therapy for neurodegenerative diseases. Cell Stem Cell. 2023 May 4;30(5):512-529. doi: 10.1016/j.stem.2023.03.017. Epub 2023 Apr 20.

[3] Agnello L, Ciaccio M. Neurodegenerative Diseases: From Molecular Basis to Therapy. Int J Mol Sci. 2022 Oct 25;23(21):12854.

[4] Maccioni, Ricardo B., Juan P. Muñoz and et al. "The molecular bases of Alzheimer's disease and other neurodegenerative disorders." Archives of medical research 32.5 (2001): 367-381.

[5] Monzio Compagnoni, Giacomo, et al. "The role of mitochondria in neurodegenerative diseases: the lesson from Alzheimer's disease and Parkinson's disease." Molecular neurobiology 57 (2020): 2959-2980.

[6] Khan, Asmat Ullah, et al. "Awareness and current knowledge of Parkinson's disease: a neurodegenerative disorder." International Journal of Neuroscience 129.1 (2019): 55-93.

[7] Ramesh, Sairam, and Arosh S. Perera Molligoda Arachchige. "Depletion of dopamine in Parkinson's disease and relevant therapeutic options: A review of the literature." AIMS neuroscience 10.3 (2023): 200.

[8] Kaye, Julia, Terry Reisine, and Steve Finkbeiner. "Huntington's disease mouse models: Unraveling the pathology caused by CAG repeat expansion." Faculty reviews 10 (2021).

[9] Bano, D., et al. "Neurodegenerative processes in Huntington's disease." Cell death & disease 2.11 (2011): e228-e228.

[10] Palmqvist S, Janelidze S, Quiroz YT. Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. JAMA. 2020 Aug 25;324(8):772-781.

[11] Thijssen EH, La Joie R, Wolf A. Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) investigators. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. Nat Med. 2020 Mar;26(3):387-397.

[12] Baiardi S, Quadalti C, Mammana A. Diagnostic value of plasma p-tau181, NfL, and GFAP in a clinical setting cohort of prevalent neurodegenerative dementias.

[13] Mielke MM, Dage JL, Frank RD. Performance of plasma phosphorylated tau 181 and 217 in the community. Nat Med. 2022 Jul;28(7):1398-1405.

[14] Gonzalez MC, Ashton NJ, Gomes BF, et al. Association of Plasma p-tau181 and p-tau231 Concentrations With Cognitive Decline in Patients With Probable Dementia With Lewy Bodies. JAMA Neurol. 2022;79(1):32–37.

[15] NfL blood test may detect neurodegeneration in youngsters.

[16] Mayer CA, Brunkhorst R, Niessner M and et al. Blood levels of glial fibrillary acidic protein (GFAP) in patients with neurological diseases. PLoS One. 2013 Apr 23;8(4):e62101.

[17] Wu, Connie, Adam M. Maley, and David R. Walt. "Single-molecule measurements in microwells for clinical applications." Critical Reviews in Clinical Laboratory Sciences 57.4 (2020): 270-290.

[18] Rivnak, Andrew J., et al. "A fully-automated, six-plex single molecule immunoassay for measuring cytokines in blood." Journal of immunological methods 424 (2015): 20-27.

[19] Wu, Connie, Padric M. Garden, and David R. Walt. "Ultrasensitive detection of attomolar protein concentrations by dropcast single molecule assays." Journal of the American Chemical Society 142.28 (2020): 12314-12323.