

NEURODEGENERATION RESEARCH.



has shown that the determination of the Amyloid-beta ratio of Amyloid-beta (1-42)/Amyloid-beta (1-40) significantly improves the understanding of amyloid-beta pathology (Figure 2). Furthermore, as fibril formation of TAU proteins is characterized by hyperphosphorylation, the determination of either phosphoTAU or the non-phosphorylated TAU fraction (Non-pTau) might help to confirm the TAU pathology in AD.

Neurofilament-Light

Neurofilaments are the backbone of the neuronal cytoskeleton. Neurofilament light is found to be elevated in neurodegenerative disorders that are associated with the destruction of white matter (substantia alba). Typically these are:

- Parkinson's disease (PD)
- Multiple Sclerosis (MS)
- Amyotrophic Lateral Sclerosis (ALS)

Prion Proteins

Several human degenerative diseases appear as a result of misfolding and aggregation of proteins. The prototype central nervous system proteinopathy is CJD, in which neuronal prion protein (PrP) with high α -helical content switches into a stable structure rich in β -pleated sheets in a self-catalyzing process that eventually causes a plethora of neurological and psychiatric symptoms.

The identification of this disease, which is extremely serious for the patient, and which shot to prominence in the bovine spongiform encephalopathy crisis, by distinguishing it from forms of dementia such as AD is a major challenge in neurochemical diagnostics. This is because atypical AD phenotypes can be presented with high levels of total Tau protein and/ or positive 14-3-3 protein

α -Synuclein

α -Synuclein is an abundant neuronal 140 amino acid protein, predominantly localized in the presynaptic terminals, and involved in vesicle fusion and neurotransmitter release. Aggregates of α -Synuclein

Parameter	Cat. No.	Method	Reg. Status
Amyloid-beta (1-40) CSF	RE59651	ELISA	CE***
Amyloid-beta (1-42) CSF	RE59661	ELISA	CE***
PhosphoTAU**	30121609	ELISA	CE***
hTAU total**	RE59631	ELISA	CE*
Non-pTAU**	RE59641	ELISA	CE***
p231 TAU**	30227873	ELISA	RUO*
TAU AGGREGATE**	30227874	ELISA	RUO*

Parameter	Cat. No.	Method	Reg. Status
Neurofilament-Light (NF-Light) CSF**	UD51001	ELISA	CE***
Neurofilament-Light (NF-Light) Serum**	30210101	ELISA	RUO*

Parameter	Cat. No.	Method	Reg. Status
BetaPrion® Human**	30227871	ELISA	RUO*

in the CSF, reflecting intense neuronal degeneration similar to what is found in CJD. The current diagnostic criterion is unfortunately characterized by a diagnostic specificity of 71 % for CJD. Ideally, an additional biomarker more closely related to the pathological process would be helpful in these cases.

Recent studies have shown that atypical cases of AD can be clearly distinguished from CJD via the detection of Prion protein in CSF samples³. The BetaPrion® human ELISA precisely enables quantification of this biomarker and may be beneficial in addition to the current classic biomarkers.

are the main components of Lewy bodies (LB), which are intracellular inclusions characteristic for certain neurodegenerative diseases referred to as α -synucleinopathies. These include Parkinson's

disease (PD), Parkinson's disease dementia (PDD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA).

However, α -Synuclein aggregates are also found in approximately half of sporadic AD pathologies; consequently, it is crucial to differentiate it from pure AD forms. Via the hSYN total ELISA, Tecan offers an improved ELISA, provided by Roboscreen, for detection of total human α -Synuclein. Additionally, the discrimination of total α -Synuclein and disease-specific α -Synuclein is of special interest for distinguishing between different patient groups.

TDP43

The transactive response region DNA-binding protein 43 (TDP43) binds both DNA and RNA and is involved in transcription and splicing. Under pathophysiological conditions, TDP43 accumulates in the cytoplasm and is hyperphosphorylated and/or ubiquitinated, and this is characteristic for the cytoplasmic inclusions observed in ALS and in many cases of frontotemporal lobar degeneration syndrome (FTLD). Furthermore, TDP43 pathology is also detected in 20-50 % of AD patients and appears

Parameter	Cat. No.	Method	Reg. Status
hSYN total**	30227869	ELISA	RUO*
α -Synuclein PATHO**	30227870	ELISA	RUO*

The Anti-human α -Synuclein 5G4, monoclonal antibody strongly binds to the high molecular weight fraction of β -sheet rich oligomers, while no binding to primarily disordered oligomers or monomers is observed. This outstanding capability is used for the human α -Synuclein PATHO ELISA suggesting a promising tool for PD.

Parameter	Cat. No.	Method	Reg. Status
hTDP43 total**	30227872	ELISA	RUO*

to be associated with greater brain atrophy, memory loss, and cognitive impairment. Several studies have been reported on CSF and plasma TDP43 in the context of ALS and FTLD, but research has been hindered by difficulties with detecting the protein. Overall, research suggests that blood based TDP43 may have a role in neurodegenerative biomarkers and could be more useful than CSF TDP43.

MEASURING NEURONAL BIOMARKERS IN BLOOD.

As CSF has disadvantages in sampling and not all patients do receive a lumbar puncture (e.g. PD, LBD... patients) studies have shown the usefulness of measuring certain biomarkers also in blood.

Boost your blood plasma data.

Easily eliminate inhibiting matrix effects and enrich your target by immunoprecipitation using TECAN's NEURO IP product portfolio provided by Roboscreen for total TAU, p50Tau, brain-derived TAU, beta-Amyloid, and patho-oligomeric alpha-Synuclein, enhancing the performance of your downstream assay of choice. The downstream analysis can then be any type of assay system, such as the Roche Elecsys®, the Mesoscale QuickPlex, Quanterix SIMOA®, Fujirebio Lumipulse® or even any type of ELISA.

Parameter	Det.	Cat. No.	Method	Reg. Status
Neuro-IP Kit**	24	30251462	IP Kit	RUO*
Brain-derived TAU**	6 IP	30251464	Immuno Beads	RUO*
Brain-derived TAU**	24 IP	30251465	Immuno Beads	RUO*
Total TAU**	6 IP	30251466	Immuno Beads	RUO*
Total TAU**	24 IP	30251467	Immuno Beads	RUO*
Human beta-Amyloid**	6 IP	30251468	Immuno Beads	RUO*
Human beta-Amyloid**	24 IP	30251469	Immuno Beads	RUO*
p50-TAU**	6 IP	30251470	Immuno Beads	RUO*
p50-TAU**	24 IP	30251471	Immuno Beads	RUO*
Patho-oligomeric α -Synuclein**	6 IP	30251472	Immuno Beads	RUO*
Patho-oligomeric α -Synuclein**	24 IP	30251473	Immuno Beads	RUO*

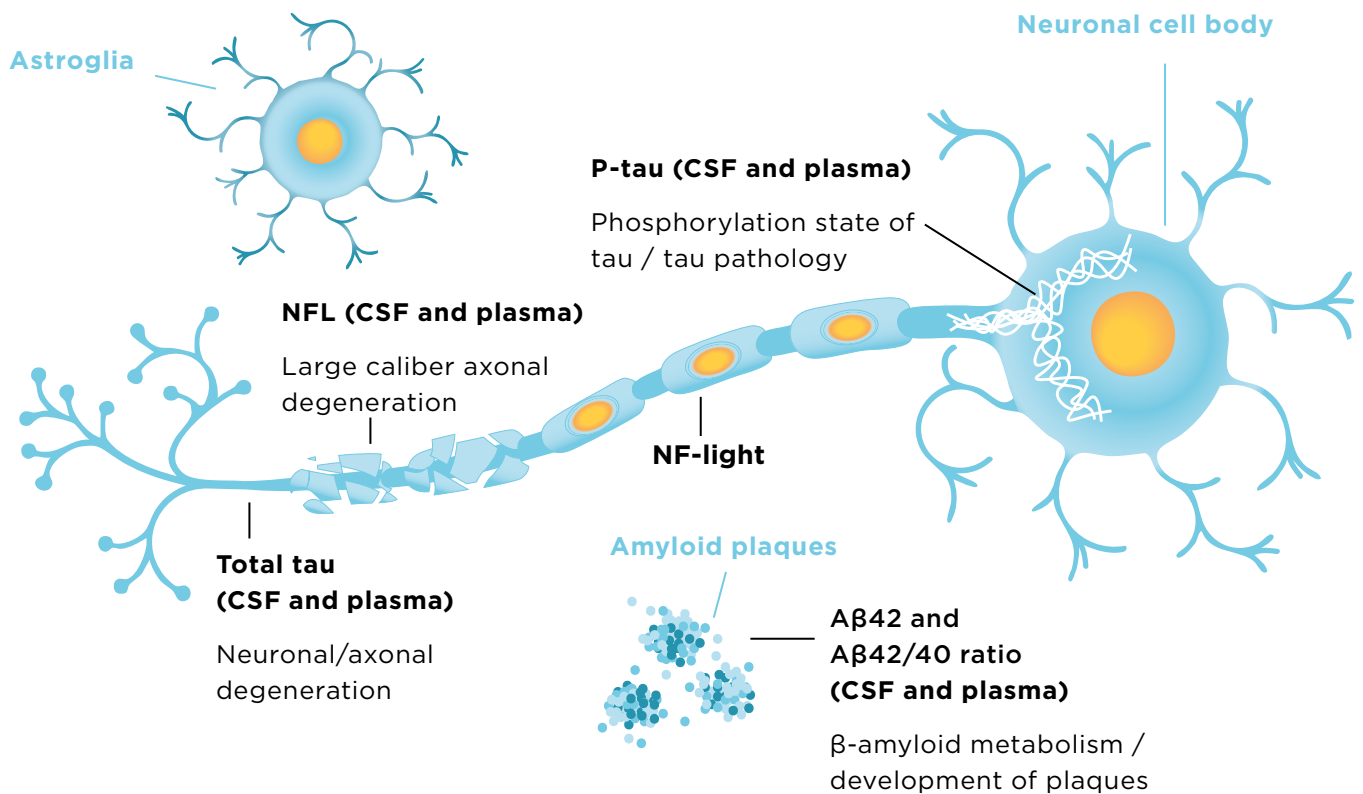
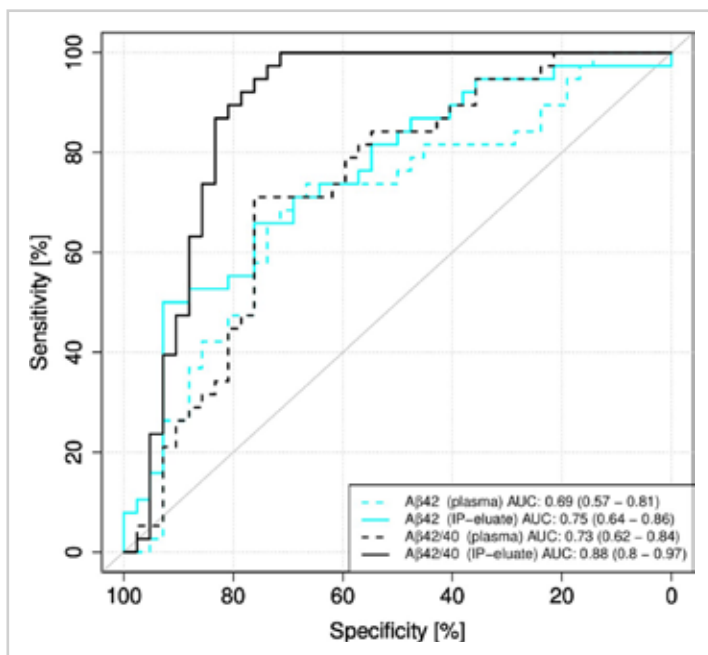


Figure 3: Overview of different biomarkers for neurodegenerative diseases and respective sample types, where they have been measured already. Adapted from O. Hansson.⁴



Elecsys®

The inclusion of pre-analytical immunoprecipitation in the plasma amyloid-β (Aβ) 42/40 ratio assay significantly improved the accuracy in identifying subjects with abnormal cerebrospinal fluid (CSF) Aβ42/40 ratios. In particular, the area under the receiver operating characteristic (ROC) curve (AUC) increased from 0.73 to 0.88 ($p = 0.01547$) when IP was applied, demonstrating a significant improvement in performance. A similar improvement was observed when a biomarker-supported clinical diagnosis was used as a secondary endpoint, with the AUC rising from 0.77 to 0.92 ($p = 0.01576$).⁵ This data strongly supports the use of immunoprecipitation in plasma-based assays for Alzheimer's disease as a means to improve accuracy in detecting amyloid biomarkers, potentially facilitating early detection and better cohort selection for clinical trials.

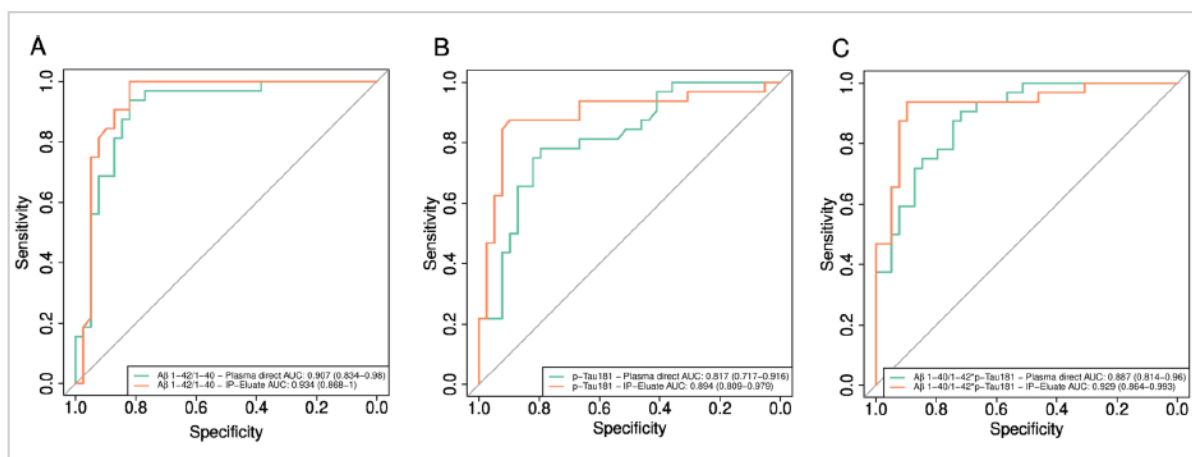
Lumipulse®

Immunoprecipitation of amyloid-beta (A β) and phosphorylated Tau (pTau) significantly improved the accuracy of these biomarkers in plasma samples.

Pre-analytical A β immunoprecipitation (IP) led to a statistically significant increase in the standardized effect size (Cohen's *d*), from 1.62 to 2.03 ($p = 0.033$), improving the differentiation between amyloid-positive and amyloid-negative individuals.

than concentrating the biomarkers. This reduction led to better contrast, as demonstrated by the improved AUCs for both A β and pTau assays.

Enhanced Specificity and Sensitivity: The pre-analytical treatment allowed for improved classification of individuals with changed amyloid condition, achieving higher sensitivity and specificity in detecting Alzheimer's biomarkers. The combined use of A β 1-42/1-40 ratios and



For phosphorylated Tau 181 (pTau181), pre-analytical Tau-IP increased the accuracy. The area under the receiver operating characteristic (ROC) curve (AUC) increased from 0.817 to 0.894 ($p = 0.039$), and the effect size (Cohen's *d*) improved from -1.17 to -1.48 ($p < 0.001$).

Matrix Effect Reduction: Pre-analytical IP was primarily aimed at reducing matrix effects rather

pTau181 after IP showed a marked increase in differential performance compared to direct plasma measurements.⁶

Overall, pre-analytical immunoprecipitation significantly enhances the value of blood-based biomarkers for Alzheimer's disease, supporting its integration into laboratory workflows.

Mesoscale

Immunoprecipitation (IP) played a critical role in the study by Klafki et al.⁷ for improving the detection of Alzheimer's disease biomarkers. Specifically, the use of IP enabled the selective pre-concentration of amyloid-beta (A β) peptides from blood plasma, which greatly enhanced the sensitivity of the subsequent immunoassay. The two-step immunoassay, which included a magnetic bead-

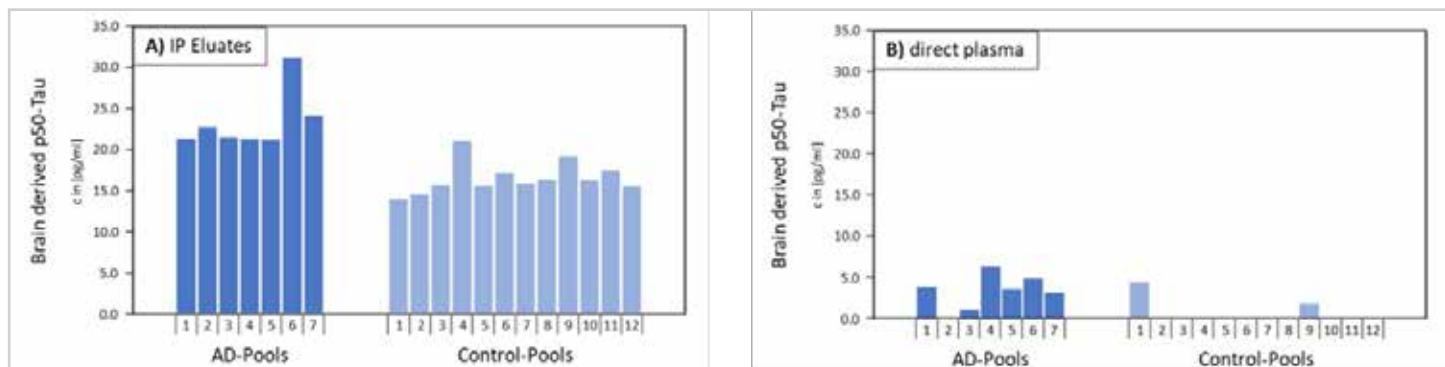
based IP followed by chemiluminescent detection, was developed to measure A β -3-40 (APP669-711), a variant of the amyloid precursor protein.

In conclusion, IP was a critical step for improving the sensitivity, specificity, and repeatability of A β peptide detection in the study, providing a strong foundation for the development of assays aimed at detecting Alzheimer's disease biomarkers.

SIMOA®

In an in-house study conducted by Roboscreen, pre-analytical Tau immunoprecipitation (IP) from blood samples enabled clear differentiation between Alzheimer's disease (AD) and control pools using a self-developed SIMOA assay for

threonine-50 phosphorylated, brain-derived TAU (p50-DB-Tau). In contrast, direct measurement without Neuro-IP could only detect 8 out of 19 plasma samples, with overlapping results between the AD and control pools.



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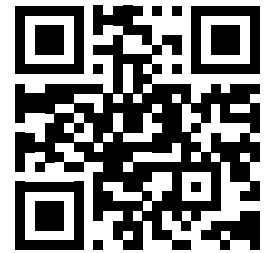
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