

Simoa® NfL Neurofilament light

What is NfL?

Neurofilament light (NfL) is a 68 kDa cytoskeletal intermediate filament protein that is highly expressed in neuronal axons. It associates with the 125 kDa Neurofilament medium (NfM) and the 200 kDa Neurofilament heavy (NfH) to form neurofilaments. Neurofilaments are major components of the neuronal cytoskeleton and function to provide structural stability to neurons as well as regulate axon diameter. Under normal conditions, neurofilaments are released into the extracellular space and further into the cerebrospinal fluid (CSF) and blood at low levels in an age-dependent manner. However, following neuro-axonal damage or neurodegeneration, release of neurofilaments significantly increases. CSF and blood NfL has been shown to be increased in patients with multiple sclerosis (MS), Alzheimer's Disease (AD), Parkinson's disease (PD), stroke, traumatic brain injury (TBI), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and other neurological disorders, positioning NfL as a promising biomarker.

How to Measure NfL?

The Simoa® NfL assay is a high sensitivity digital immunoassay for the quantitative determination of NfL in serum, plasma and CSF. The antibodies (Uman Diagnostics, Umeå Sweden) also cross react with murine, bovine, and macaque NfL epitopes, and the assay can be used for research with these species. Quanterix also offers Simoa® NfL testing in human serum only as a Laboratory Developed Test (NfL-LTD) that has been validated under CLIA. This test is not currently cleared by the U.S. FDA as an in vitro diagnostic.

What is the Simoa® Difference?

Simoa® is a powerful digital immunoassay technology that is up to 1000 times more sensitive than standard sandwich-based immunoassay techniques. Traditional ELISA measurements are limited to pg/ml levels of detection. Quanterix Simoa® can achieve sensitivity as low as femtogram (fg/ml) levels, delivering the gold standard for early, ultra-sensitive detection and quantification of proteins far below the typical lower limit of quantification (LLOQ).

Simoa® is based upon the isolation of individual immunocomplexes on paramagnetic beads using standard ELISA reagents. The main difference between Simoa® and conventional immunoassays lies in the ability to trap single molecules in femtoliter-sized wells, allowing for a "digital" readout of each individual bead to determine if it is bound to the target analyte or not.

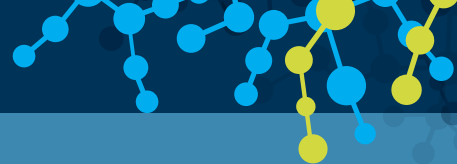
Simoa® NfL testing is offered by Quanterix as a lab developed test (NfL-LDT) per CLIA and CLSI guidelines. Quanterix's state-of-the-art facility includes a CLIA-licensed lab and supports over 400 customers globally. With the usage of large normative reference range databases, the Simoa NfL-LTD test has been adopted for clinical use in several applications.

Neurological disorders continue to be among the most difficult to diagnose early and treat. Unlike "visible" illnesses, neuronal injury and neurodegeneration can be overlooked or mistaken for other conditions. The subtlety of symptoms and subjective nature of today's assessments also make it difficult to identify these diseases early. Currently, no definitive tests exist for early detection of neurodegenerative diseases such as MS, AD and PD. Clinicians can only conclusively diagnose them once symptoms start to present. As a result, many patients may wait years for a diagnosis or clear treatment pathway.

NfL has emerged in recent years as a blood-based biomarker with the potential to aid in the improvement of early diagnosis and patient care of neuro-axonal injury and neurodegeneration. Hundreds of studies using Simoa® have validated the use of NfL as a promising and critical tool for early detection, prognosis and monitoring treatment for a range of neurodegenerative conditions.



NfL in Action



Blood NfL as a biomarker of MS disease activity and treatment response

The study assessed blood NfL as a biomarker of disease activity and its utility to monitor treatment response in relapsing-remitting multiple sclerosis (RRMS). NfL was measured in blood samples from 589 patients with RRMS and 35 healthy controls. Blood NfL levels were shown to be associated with clinical and MRI-related measures of disease activity and neuroaxonal damage and have prognostic value. The results support the utility of blood NfL as an easily accessible biomarker of disease evolution and treatment response.

Kuhle J, Kropshofer H, Haering DA, et al. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. *Neurology* 2019;92(10):e1007-e1015. doi.org/10.1212/WNL.0000000000007032

NfL reveals causal link between Epstein-Barr virus (EBV) and MS

The underlying cause of MS is not known, but EBV is thought to be a possibility. The study measured serum NfL levels from 810 MS patients and 1577 matched controls over time. Serum NfL levels increased in MS patients only after EBV infection, indicating that EBV infection preceded MS symptom onset and all other detectable pathological mechanisms underlying MS. These findings suggest EBV as the leading cause of MS.

Bjornevik K, Cortese M, Healy BC, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science*. 2022;375(6578):296-301. doi.org/10.1126/science.abj8222

Plasma NfL as a promising biomarker panel to differentiate Alzheimer's disease from frontotemporal dementia (FTD) and dementia with Lewy bodies (DLB)

This study explored what combination of blood-based biomarkers could differentiate AD dementia, FTD, and DLB. Plasma NfL, in addition to amyloid beta (Aβ)40/42, phosphorylated tau 181 (p-tau181), and glial fibrillary acidic protein (GFAP), were measured in two separate cohorts (n = 160 and n = 152). MS and Simoa Aβ1-42/1-40 similarly differentiated AD from controls. NfL, GFAP and p-tau181 differentiated AD from FTD and DLB, suggesting the combination as useful biomarkers.

Thijssen EH, Verberk IM, Kindermans J, et al. Differential diagnostic performance of a panel of plasma biomarkers for different types of dementia. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*. 2022;14(1):e12285. doi.org/10.1002/dad2.12285

Serum NfL levels predict progression of symptoms in patients first diagnosed with Parkinson's disease

In PD, NfL levels are associated with disease severity, risk of progression, and survival. This study examined whether baseline serum NfL levels at the time of PD diagnosis can predict symptom progression. Serum NfL was measured in 376 de novo PD patients. Patients were monitored for 8 years to assess motor disability, postural instability gait disorder (PIGD) versus tremor clinical features, cognitive function, change in dopamine transporter (DAT) uptake over time. Elevated NfL at time of diagnosis were associated with increased motor decline, PIGD, cognitive decline, and DAT loss. These findings suggest that baseline serum NfL levels predict the rate of symptom progression in the early stage of PD.

Ye R, Locascio JJ, Goodheart AE, et al. Serum NfL levels predict progression of motor impairment and reduction in putamen dopamine transporter binding ratios in de novo Parkinson's disease: an 8-year longitudinal study. *Parkinsonism & related disorders*. 2021;85:11-6. doi.org/10.1016/j.parkreidis.2021.02.008

Plasma NfL predicts mortality in patients with stroke

This study evaluated whether blood NfL could serve as a biomarker to determine the degree of neuroaxonal injury across stroke types. Blood NfL was measured in patients with acute cerebral infarction (ACI; N = 227), aneurysmal subarachnoid hemorrhage (aSAH; N = 58), and nontraumatic intracerebral hemorrhage (ICH; N = 29). Compared to healthy individuals, NfL levels were elevated for all stroke types and associated with radiographic markers of brain tissue damage. The results support the use of NfL to estimate neuroaxonal injury and predict mortality across stroke types.

Gendron TF, Badi MK, Heckman MG, et al. Plasma neurofilament light predicts mortality in patients with stroke. *Science translational medicine*. 2020;12(569):eay1913. doi.org/10.1126/scitranslmed.aay1913

NfL as a biomarker in traumatic brain injury

This study aimed to determine whether serum NfL correlates with CSF NfL, TBI diagnosis, injury severity, brain volume, and diffusion tensor imaging (DTI) estimates of traumatic axonal injury (TAI). CSF NfL and serum NfL were correlated in patients with TBIs. NfL levels could distinguish patients with mild TBI from those with moderate and severe TBI and correlated with measures of functional outcome, MRI brain atrophy, and DTI estimates of traumatic axonal injury. This study demonstrates serum NfL as a promising biomarker for acute and repetitive sports-related concussion and patients with subacute and chronic TBI.

Shahim P, Politis A, van der Merwe A, et al. Neurofilament light as a biomarker in traumatic brain injury. *Neurology*. 2020;95(6):e610-22. doi.org/10.1212/WNL.0000000000009983

NfL in the News

FDA Accelerated Approval of Tofersen Highlights Importance of Blood NfL as Surrogate Endpoint in Neurology Therapeutic Trials

In April 2023, blood-based NfL measurements provided compelling support for the FDA's accelerated approval of tofersen for treatment of superoxide dismutase 1 amyotrophic lateral sclerosis (SOD1-ALS), a devastating rare genetic form of ALS. This is the first known case in which a blood biomarker was successfully used as a surrogate endpoint for a neurology therapeutic trial to gain accelerated approval, highlighting the potential for other therapeutic trial designs to benefit from including blood NfL measurements.

Critical to the approval were plasma NfL trends, with the FDA Advisory Committee voting unanimously that the "reduction in plasma neurofilament light chain (NfL) concentration in tofersen-treated patients is reasonably likely to predict clinical benefit of tofersen for treatment of patients with SOD1-ALS." Tofersen was found to lower plasma NfL levels 40-50 percent over a six-month period.

"An important advance stemming from the tofersen approval is a demonstration of the potential of NfL as a potential surrogate biomarker that can serve as a leading indicator of drug efficacy for some investigational therapies in neurodegenerative disorders," said Merit Cudkowicz, M.D., M.Sc., Director of the Sean M. Healey & AMG Center for ALS and Chair of Neurology at Mass General. "The acceptance of NfL as a valid biomarker for accelerated therapeutics in ALS is a major advance. I fully expect that more biomarkers like NfL will be discovered in ALS. This is a very exciting and hopeful time in ALS therapeutics."

