## Overview

### Useful For
Assessing neuronal damage related to various neurodegenerative diseases

### Method Name
Chemiluminescent Immunoassay

### NY State Available
Yes

### Reporting Name
Neurofilament Light Chain, P

### Aliases
NfL

## Specimen

### Specimen Type
Plasma

### Specimen Required
**Collection Container/Tube:** Lavender top (EDTA)
**Submission Container/Tube:** Plastic vial
**Specimen Volume:** 1 mL
**Collection Instructions:** Centrifuge and aliquot plasma into a plastic vial. Do not submit in original tube.

### Specimen Minimum Volume
0.75 mL

### Reject Due To

| | |
|---|---|
| Gross hemolysis | Reject |
| Thawing | Cold ok; Warm reject |
| Gross lipemia | Reject |
| Gross icterus | Reject |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Plasma EDTA | Frozen(preferred) | 90 days | |
| | Refrigerated | 14 days | |

## Clinical & Interpretive

### Clinical Information
Neurofilaments (NF) are exclusively located in the neuronal cytoskeleton and are released to the interstitial fluid upon axonal injury or neurodegeneration. NF concentrations in cerebrospinal fluid (CSF) and blood have been shown to

correlate with the extent of axonal damage or neurodegeneration in various neurodegenerative diseases. Of the family of NF proteins, neurofilament light chain (NfL) has gained the most interest as a candidate marker of neurodegeneration. During axonal damage, NfL is released into the CSF, and eventually into the blood where concentrations are 40-fold lower than in the CSF. Concentrations of NfL in plasma have been shown to be approximately 5% to 10% lower than those measured in serum.

Circulating NfL concentrations increase with age with reported increases of approximately 2% to 3% per year in both male and female individuals. While the specific cause of this increase has not been elucidated, it is believed to be related to the aging process as well as to the development of subclinical ischemic events. NfL concentrations in blood (plasma or serum) reflect the extent of axonal damage, making them a generic marker of disease activity. Increases in NfL concentrations have been reported in individuals with traumatic brain injury, amyotrophic lateral sclerosis, multiple sclerosis, frontotemporal dementia, Alzheimer disease, and other neurodegenerative diseases.

### Reference Values

<2.5 years: < or =12.8 pg/mL
2.5 to 4 years: < or =11.8 pg/mL
5 to 9 years: < or =10.4 pg/mL
10 to 14 years: < or =8.8 pg/mL
15 to 19 years: < or =9.2 pg/mL
20 to 24 years: < or =10.4 pg/mL
25 to 29 years: < or =11.9 pg/mL
30 to 34 years: < or =13.5 pg/mL
35 to 39 years: < or =15.3 pg/mL
40 to 44 years: < or =17.3 pg/mL
45 to 49 years: < or =19.7 pg/mL
50 to 54 years: < or =22.4 pg/mL
55 to 59 years: < or =25.4 pg/mL
60 to 64 years: < or =28.8 pg/mL
65 to 69 years: < or =32.7 pg/mL
70 to 74 years: < or =37.1 pg/mL
75 to 79 years: < or =42.1 pg/mL
80 to 84 years: < or = 47.8 pg/mL
> or =85 years: < or =54.3 pg/mL

### Interpretation

Blood neurofilament light chain (NfL) is a non-specific marker of neuro-axonal injury showing promising associations with outcomes in several neurological conditions. In neurodegenerative diseases, NfL may also serve as a prognostic marker of decline and an efficacy biomarker of experimental therapies. In a meta-analysis of Alzheimer disease (AD), frontotemporal dementia, and amyotrophic lateral sclerosis (ALS), plasma NfL levels were elevated in patients compared to controls with utility in differentiating neurodegenerative conditions from non-neurodegenerative mimics. However, due to a lack of specificity to a particular neurodegenerative disease, its role as a diagnostic marker is limited.

In multiple sclerosis (MS), NfL is elevated in the blood of newly diagnosed patients and concentrations correlate with disease severity and prognosis. However, measured circulating NfL in patients with MS often overlaps with concentrations found in healthy individuals, potentially limiting its utility. Early measures of blood NfL in patients newly diagnosed with MS can predict brain atrophy and lesion load on magnetic resonance imaging. The use of blood NfL in serial disease monitoring and treatment response has been evaluated in various prospective clinical trials. Reductions in NfL concentrations after different treatments tend to follow the hierarchy of treatment efficacy, with greatest reductions observed with the most intensive treatments. A study that included over 1000 patients with MS receiving various treatments, reported the largest reductions in plasma NfL concentrations following alemtuzumab treatment (54% reduction), and the smallest reduction with teriflunomide treatment (7%).

Pediatric-onset MS prevalence and incidence rates are increasing globally. Between 3% and 10% of patients with MS

present under 16 years of age. Guidelines for pediatric MS recommend that treatment can be started early in the disease course. Circulating NfL is a useful biomarker for monitoring disease activity and treatment response in pediatric MS. It is most likely helpful to predict disease severity and to guide treatment decisions in patients with pediatric MS. Elevated blood NfL levels were significantly associated with higher numbers of cerebral and spinal MRI lesions at baseline. High concentrations of blood NfL (>100 pg/mL) are predictive of disease events within four months.

In AD, blood NfL concentrations have been shown to correlate with cortical thinning and cognitive decline in both sporadic and familial AD. While there are elevations of NfL in patients with AD, at this point the clinical value of blood NfL is not fully understood, especially in light of the availability of other more sensitive and specific biomarkers for AD.

Circulating NfL has been evaluated both in the context of mild traumatic brain injury (mTBI) diagnosis (acute setting) and prognosis (outcome prediction). In the acute setting, the utility of NfL in identifying mTBI within 24 hours of an injury has been controversial, likely due to the different timepoints used in studies for evaluating NfL concentrations after the injury (ranging from 1-, 4-, 6-, 12-, and 24-hours post-injury). A recent 2022 review describes the findings of six different publications looking at the role of NfL in acute mTBI. Three out of four studies involving athletes reported data supporting the efficacy of NfL in identifying mTBI within 24 hours of an injury. However, studies using either military cadets or emergency department patients presenting with possible mTBI as subjects did not show evidence supporting NfL use in the acute setting. As a prognostic marker, data has been inconclusive as to whether NfL is able to predict recovery time, post-concussion syndrome, or potential return to sport after mTBI.

In ALS, NfL concentrations have been suggested to be able to discriminate ALS from ALS-mimics. NfL levels at symptom onset may be prognostic of disease progression rate and may be used to stratify patients into groups with a similar prognosis in clinical trials. Blood NfL concentrations remain relatively stable throughout the disease. The US Food and Drug Administration has approved Qalsody (tofersen) to treat patients with ALS associated with a genetic variant in the superoxide dismutase 1 (SOD1) gene. The approval was based on a reduction in plasma NfL at the end treatment compared to the placebo arm. Therefore, NfL might be a useful biomarker of assess effectiveness of some ALS therapies.

Parkinson disease (PD) patients with elevated NfL concentrations have been reported to have worse cognitive decline, brain cortical atrophy, and motor scores. Blood NfL concentrations in atypical forms of Parkinson disease are higher than in PD and may be used to help differentiate PD from atypical parkinsonian disorders.

In frontotemporal dementia (FTD), blood NfL was able to discriminate patients with the behavioral form of FTD from patients with primary psychiatric disorders. It has been suggested that blood NfL could be used to support the diagnosis of the behavioral form of FTD, monitor disease progression, and prognosis of FTD.

### Cautions

Increases in neurofilament light chain (NfL) are not disease specific. Results should only be used in conjunction with other clinical information when evaluating patients with neurodegeneration.

Higher concentrations of NfL may be found in persons with history of stroke, atrial fibrillation, myocardial infarction, chronic kidney disease, pregnancy, and diabetes.

Lower concentrations of NfL may be found in individuals with a BMI of 30 or more.

All immunometric assays can, on rare occasions, be subject to a hooking effect at extremely high analyte concentrations (false-low results), heterophilic antibody interference (false-high results), or autoantibody interference (unpredictable effects). If the laboratory result does not fit the clinical picture, these possibilities should be considered.

### Clinical Reference

1. Bornhorst JA, Figdore D, Campbell MR, et al. Plasma neurofilament light chain (NfL) reference interval determination in an age-stratified cognitively unimpaired cohort. Clin Chim Acta. 2022;535:153-156. doi:10.1016/j.cca.2022.08.017

2. Barro C, Chitnis T, Weiner HL. Blood neurofilament light: a critical review of its application to neurologic disease. Ann Clin Transl Neurol. 2020;7(12):2508-2523.
3. Ashton NJ, Janelidze S, Al Khleifat A, et al. A multicentre validation study of the diagnostic value of plasma neurofilament light. Nat Commun. 2021;12(1):3400
4. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol. 2018;14(10):577-589
5. Farragher CD, Ku Y, Powers JE. The potential role of neurofilament light in mild traumatic brain injury diagnosis: a systematic review. Cureus. 2022;14(11):e31301. doi:10.7759/cureus.31301
6. Disanto G, Barro C, Benkert P, et al. Serum neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2017;81(6):857-870
7. Wendel EM, Bertolini A, Kousoulos L, et al. Serum neurofilament light-chain levels in children with monophasic myelin oligodendrocyte glycoprotein-associated disease, multiple sclerosis, and other acquired demyelinating syndrome. Mult Scler. 2022;28(10):1553-1561
8. Verde F, Steinacker P, Weishaupt JH, et al. Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2019;90(2):157-164
9. Mielke MM, Syrjanen JA, Blennow K, et al. Plasma and CSF neurofilament light: Relation to longitudinal neuroimaging and cognitive measures. Neurology. 2019;93(3):e252-e260
10. Khalil M, Pirpamer L, Hofer E, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nat Commun. 2020;11(1):812

## Performance

### Method Description
The Lumipulse G NfL Blood is an assay system for the quantitative measurement of neurofilament light chain (NfL) in plasma specimens based on chemiluminescent immunoassay technology by a specific two-step sandwich immunoassay method on the Lumipulse G System. The specimen and biotinylated antibody solution are both added to the antibody coated particle solution. The NfL in the specimen specifically binds to anti-NfL monoclonal mouse antibody on the particles and biotinylated mouse antibody. Biotinylated antibody-antigen immunocomplexes are formed. The particles are washed and rinsed to remove unbound materials. Alkaline phosphatase labeled streptavidin specifically binds to biotinylated immuno-complexes on the particles. The particles are washed and rinsed to remove unbound materials. Substrate solution is added and mixed with the particles. 3-(2'-Spiroadamantyl)-4-methoxy-4-(3"-phosphoryloxy)-phenyl-1,2-dioxetane (AMPPD) contained in the substrate solution is dephosphorylated by the catalysis of alkaline phosphatase indirectly conjugated to particles. Luminescence (at a maximum wavelength of 477 nm) is generated by the cleavage reaction of dephosphorylated AMPPD. The luminescent signal reflects the amount of NfL present in the sample.(Package insert: Lumipulse G NfL Blood. Fujirebio Inc; ver 1, 01/2023)

### Day(s) Performed
Tuesday. Days performed may be flexible if samples are scheduled to arrive in a batch.

### Report Available
1 to 9 days

### Specimen Retention Time
90 days

### Performing Laboratory Location
Rochester

### CLIA Laboratory Number
24D1040592

### Test Classification
For research use only. This test was developed and its performance characteristics determined by Mayo Clinic in a

manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.