Neurofilament (pNf-H) ELISA

- **Detection of pNf-H (phosphorylated neurofilament heavy chain) in CSF or serum**
- **Ready-to-use reagents**
- **Incubation time of only 135 minutes / same procedure for CSF and serum**
- **Colour change after adding calibrators and samples**

Technical data

Coating	Polyclonal anti-pNf-H antibody
Calibration	Quantitative, in nanograms per milliliter (ng/ml) 6 calibrators, defined target values
Sample dilution	CSF, serum, plasma; 25 µl; undiluted
Reagents	Ready for use, with the exception of the wash buffer (10 x); colour-coded solutions
Test procedure	120 min / 15 min (sample / substrate incubation), room temperature with shaking, fully automatable
Measurement	450 nm, reference wavelength between 620 nm and 650 nm
Test kit format	96 break-off wells; kit includes all necessary reagents
Order no.	**EQ 6561-9601**

Clinical significance

Motor neuron diseases (MND) belong to the group of neurodegenerative diseases that are characterised by degeneration of the upper and lower motor neurons. The prevalence of amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig syndrome, is 2/100,000. Thus, ALS is the most frequent MND, before primary lateral sclerosis, progressive muscular atrophy and pseudobulbar palsy. The main cause of these diseases is still unknown, despite intensive research. MND often starts with slight unspecific symptoms, such as muscle weakness or cramps in the arms and legs or dysphagia and dysarthria, depending on the affected areas of the body. When the disease progresses, the symptoms occur all over the body and increase in intensity. Finally, the patient experiences a complete loss of autonomy and the ability to communicate. Respiratory failure frequently leads to death two to four years after the start of the disease. Only 5 to 10 % of patients survive for more than ten years after the onset of the first symptoms.

Diagnostic application

ALS diagnostics are based on the assessment of clinical symptoms and on electromyography (EMG). Between the onset of the first symptoms and diagnosis there are usually >12 months. For this reason, there is an urgent need for methods for early diagnosis, for instance, imaging procedures or biochemical laboratory assays. Diagnostic assays are also used for the differentiation of ALS from other MND mimics, such as polyneuropathy, myopathy or sporadic inclusion body myositis. Recent publications show that increased values of phosphorylated neurofilament heavy chain (pNf-H) in CSF or serum could be helpful for diagnosis, differential diagnosis and prognosis in MND. It is recommended to include this marker in MND routine diagnostics.

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Detection limit

The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest clearly detectable pNf-H concentration. The lower detection limit of the Neurofilament (pNf-H) ELISA from different runs is on average 0.027 ng/ml. The functional sensitivity, defined as the lowest concentration in a CSF sample with a variation coefficient of <20 %, was 0.117 ng/ml.

Linearity

The linearity of the Neurofilament (pNf-H) ELISA was determined by diluting three CSF samples (3.2–11.4 ng/ml) and three serum samples (2.1–12.6 ng/ml) in nine steps up to a final dilution of 1:10 with sample buffer. The mean concordance for the CSF samples was between 88 and 119 %, with a mean correlation coefficient of r = 0.995. The mean concordance for the serum samples was between 84 and 124 %, with a mean correlation coefficient of r = 0.983.

Reproducibility

The reproducibility of the test was investigated by determining the intra-assay, inter-assay and inter-lot coefficients of variation (CV) using three samples.

CSF	Intra-assay precision, n=20			Inter-assay precision, n = 10 x 3			Inter-Lot precision, n=3x4x2	
No.	Mean value (ng/ml)	CV (%)	No.	Mean value (ng/ml)	CV (%)	No.	Mean value (ng/ml)	CV (%)
1	0.8	3.4	1	0.8	4.5	1	0.8	9.0
2	5.5	3.1	2	5.2	7.7	2	5.0	6.1
3	7.9	3.7	3	7.9	7.1	3	7.7	5.0

Serum	Intra-assay precision, n=20			Inter-assay precision, n = 10 x 3			Inter-Lot precision, n=3x4x2	
No.	Mean value (ng/ml)	CV (%)	No.	Mean value (ng/ml)	CV (%)	No.	Mean value (ng/ml)	CV (%)
1	0.6	2.6	1	0.7	4.5	1	0.7	11.6
2	2.1	3.1	2	2.5	8.5	2	0.8	10.9
3	3.3	2.5	3	2.9	11.0	3	2.9	12.2

Expected values

Fig. 1: Clinically characterised CSF samples from 80 patients with amyotrophic lateral sclerosis (ALS), 21 patients with another neurological disease (DC) and three healthy control persons (HC) were analysed using the Neurofilament (pNf-H) ELISA. The median of all ALS patients was 2.12 ng/ml, whereas the median values for the two control panels were 0.43 ng/ml (DC) and 0.17 ng/ml (HC).

Fig. 2: Clinically characterised serum samples from 20 patients with amyotrophic lateral sclerosis (ALS), 29 patients with another neurological disease (DC) and 14 healthy control persons (HC) were analysed using the Neurofilament (pNf-H) ELISA. The median of all ALS patients was 0.57 ng/ml, whereas the values for both control panels were 0.02 ng/ml.

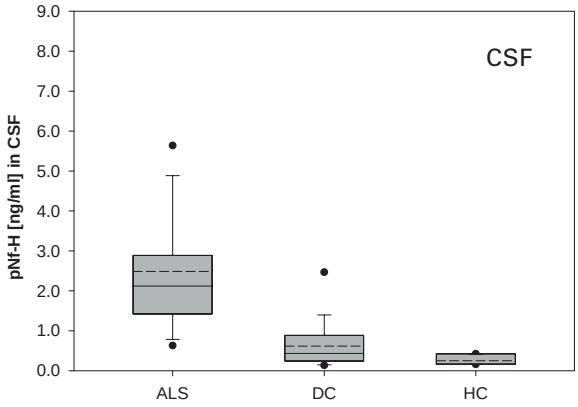

Fig. 1

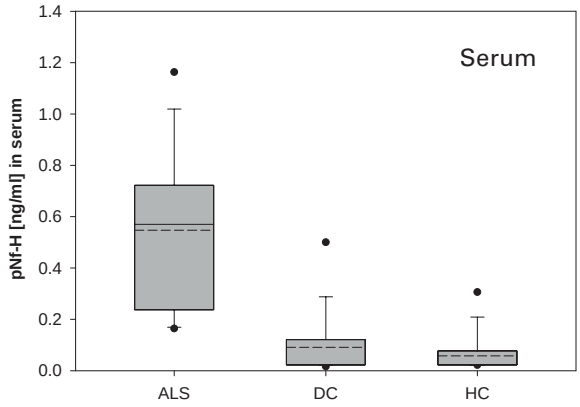

Fig. 2

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EQ_6561_D_UK_A01, 08/2018