

Table 1: Patient characteristics

	Controls	ALS
n	5	5
Sex (f/m)	3/2	4/1
Age (years) ¹	62 (47-75)	69 (60-79)
Disease onset (spinal/bulbar)	N/A	2/3
Disease duration (months) ¹	N/A	9(6.5-11.5)
ALS-FRS-r	N/A	37 (29-41)

ALS, amyotrophic lateral sclerosis; f, female; m, male

¹median and interquartile range

Table 2: Details of Assays Characteristics

Assay Platform	Analyte	Assay Format	Biofluid	Assay Performance	Reference
Simoa	NfL	Single Molecule Array Technology	Serum, Plasma, CSF	LLOD – 0.038pg/ml LLOQ – 0.174pg/ml	
Simoa (In-house)	NfL	Single Molecule Array Technology	Serum, Plasma, CSF	Analytical sensitivity – 0.32pg/ml	[11]
Simoa (In-house)	NfL	Single Molecule Array Technology	Plasma	LLOQ 2.3pg/ml	[15]
IBL International	NfL	2 site solid phase sandwich ELISA	CSF	Detection Limit – 33pg/ml	
MesoScale Discovery (In-house)	NfL	Electrochemiluminescence (ECL)	Serum	15.6pg/ml	[14]
In-house ELISA	NfL	ELISA	CSF	LLOQ 78pg/ml	[12]
Euroimmun	pNfH	ELISA	Serum, Plasma, CSF	Serum 6pg/ml CSF 20pg/ml	
Simoa	pNfH	Single Molecule Array Technology	Serum, Plasma, CSF	LLOD – 0.663pg/ml LLOQ – 2.88pg/ml	
MesoScale Discovery (Iron Horse)	pNfH	Electrochemiluminescence (ECL)	Serum, Plasma		
Luminex	pNfH	xMAP technology	CSF	LLOD 6.6pg/ml LLOQ 7.8pg/ml	[16]
BioVendor	pNfH	ELISA	CSF	LOD 23.5pg/ml	

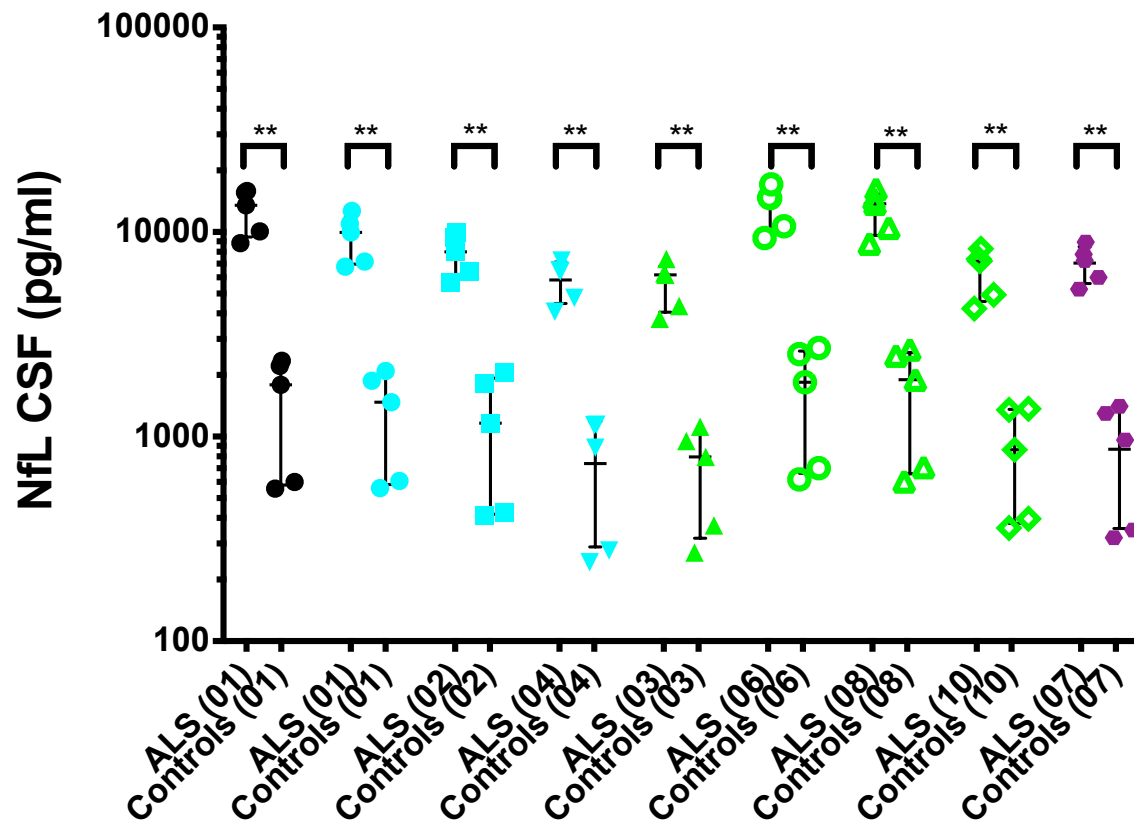


Figure 1

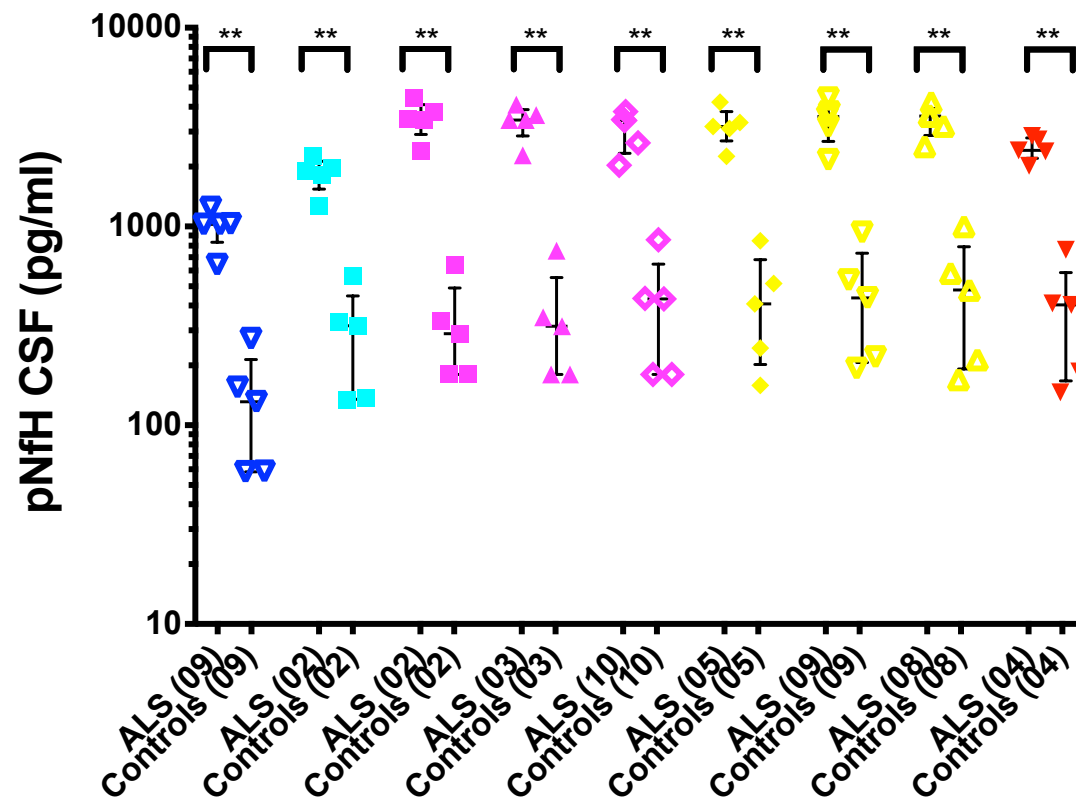


Figure 2

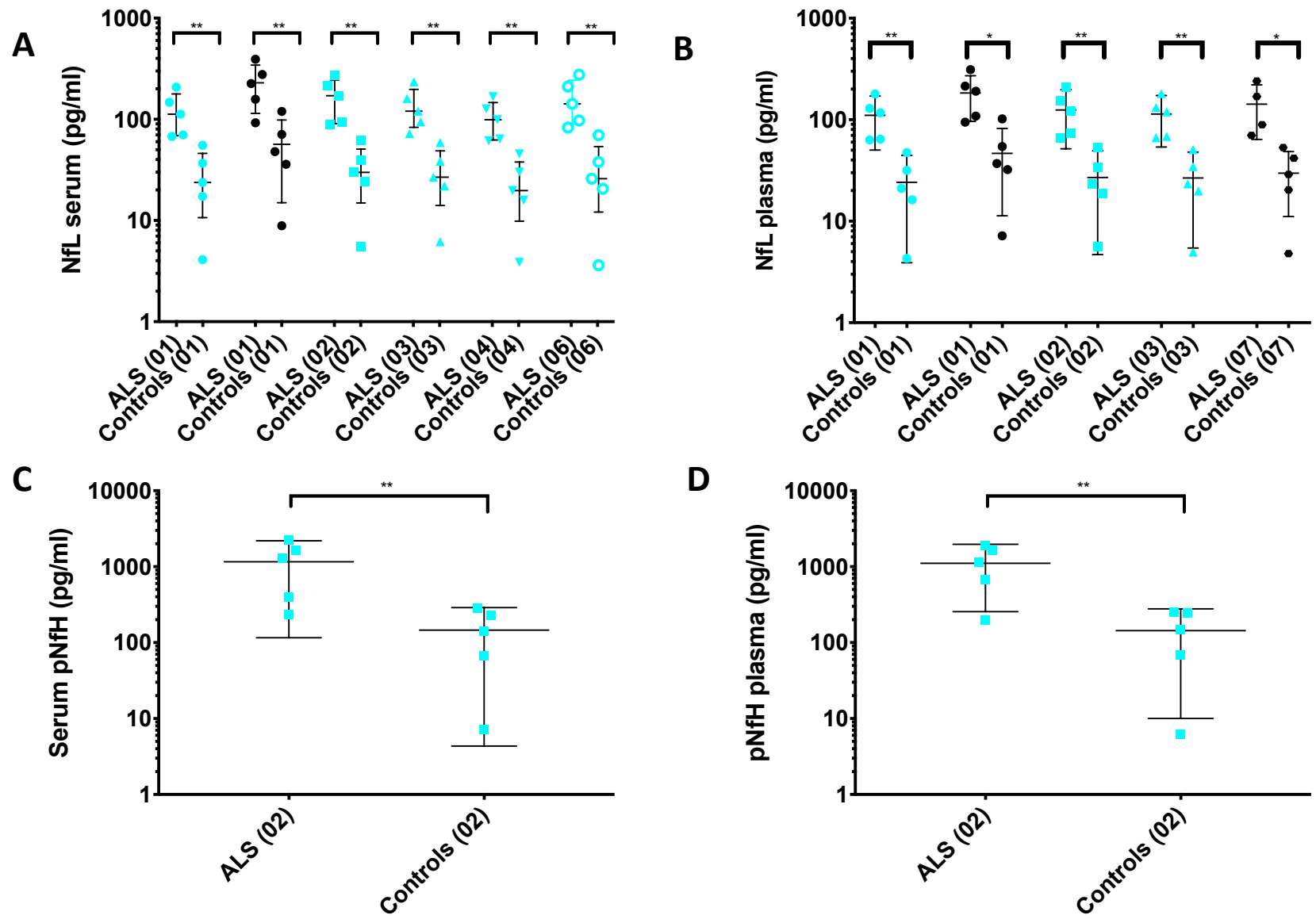


Figure 3

	Simoa NfL assay	EuroImmun pNfH ELISA	Uman NfL ELISA	BioVendor pNfH
Serum	18.3±4.2	17.6±9.5	-	-
Plasma	7.18±3.4	18.5±12.2	-	-
CSF	27.9±6.8	6.5±2.8	41.9±3.4	11.5±6.1

Table 3: Assessment of inter-laboratory assay reliability For each analytical platform that was used across multiple laboratories; the coefficient of variation was calculated for each of the 10 samples measured (per bio-fluid sample type). The inter-laboratory coefficient of variation was then calculated from these 10 data points (mean± SD% CV)