Validation of Nf-L in Human Matrix using the Quanterix HD-X

May 2020

Validation of Nf-L in Human Matrix using the Quanterix HD-X

LGC has successfully completed a validation of Neurofilament-Light (Nf-L) in healthy human serum and plasma on one of Europe's first fully validated and compliant HD-X systems

Summary of System

- **Ultra-sensitive, sub pg/mL levels of detection**
- **High throughput analysis of >500 samples per day**
- **Fully validated methods performed in less than two weeks**
- **Customisable assay format with fast development potential**
- **Multiplexing capabilities of up to 6 biomarkers**
- **Biomarker/PD and PK applications**
- **A wide range of >80 biomarker and seven multiplex kits available**

Summary of Validation

- **Validation of healthy plasma and serum completed in nine days (pending LTS)**

Parameter Assessed	Outcome	Successful
Six P&A runs using three analysts	Inter-assay precision of ~5% across five QC levels	✓
Curve and weighting assessment	Statistical assessment of curve fit on six P&A runs concluded that a 5PL 1/Y2 weighting was optimal	✓
Suitability for singlicate assessment	Singlicate analysis can be completed on both serum and plasma	✓
Multiplate analysis	Instrument can run at full capacity (288 samples) across the validated range with acceptable precision and accuracy	✓
Kit lot to lot variation	Lot to lot assessment completed and minimal bridging will be required when changing kit lots during sample analysis studies	✓
6 X freeze/thaw and 2hr room temperature stability	Both assessment acceptable with minimal variation seen	✓
Matrix effects – Haemolysed and Lipaemic samples	No evidence of matrix effects from endogenous QCs at expected sample concentrations	✓
Parallelism of endogenous samples	Acceptable parallelism of 2-fold in both serum and plasma. This can be extended with incurred samples. All samples expected to come in with MRD (4-fold)	✓
LTS of up to one year pending	To be tested at one month, three months and one year at both -80°C and -20°C	Pending

What this method offers:

- Nf-L analysis for both healthy human serum and plasma samples across a wide dynamic range on a fully validated and compliant system
- Potential for up to 2500 samples per week analysed in our laboratory
- Minimal to no failures expected as a result of high kit reproducibility and platform reliability
- Minimal hands on time required so data processing timelines reduced
- Dedicated high capacity sample management and logistics team

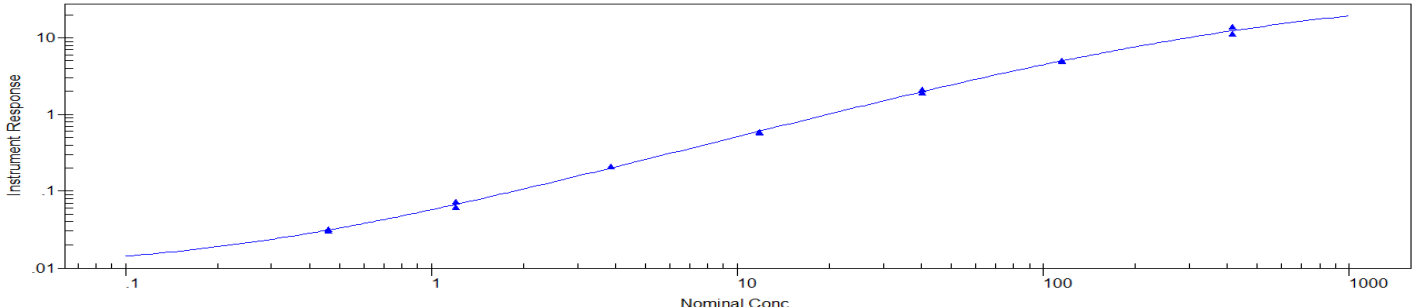
Validation Results

Nf-L calibrator results:

Calibration model:	
Fit	5 parameter (auto estimate)
Weighting	$1/Y^2$
Assay calibration range	Kit lot specific: 502183: 0.461 to 415 pg/mL 501998: 0.471 to 453 pg/mL 502186: 0.525 to 561 pg/mL
Sample volume	334 µL
Upper limit of quantitation	123 pg/mL (Serum ULQC), 106 pg/mL (Plasma ULQC). Upper calibration range is kit specific.
Lower limit of quantitation	2.34 pg/mL (Serum LLQC), 2.34 pg/mL (Plasma LLQC), Lower calibration range is kit specific.
MRD in sample diluent	4 fold (performed on instrument)

Nf-L calibrator performance:

	Cal B	Cal C	Cal D	Cal E	Cal F	Cal G	Cal H
pg/mL	**0.461**	**1.20**	**3.87**	**11.8**	**40.2**	**115**	**415**
Mean Concentration Found (pg/mL)	0.467	1.19	3.94	11.9	40.1	115	419
Inter-run %CV	6.2	6.2	3.8	3.4	5.1	3.6	7.6
Inter-run %RE	1.3	-0.8	1.8	0.8	-0.2	0	1
n	32	32	32	32	32	30	32



Concentrations pg/mL

Serum and Plasma QC P&A Performance:

QC	Serum conc (pg/mL)	Intra %CV	Inter %RE	Inter %CV	Plasma conc (pg/mL)	Intra %CV	Inter %RE	Inter %CV
LLQC	2.34	≤ 9.6	-3.8 to 7.7	≤ 6.2	2.34	≤ 9.6	-5.6 to 3.4	≤ 6.3
LQC	5.34	≤ 6.9	-5.8 to 8.1	≤ 6.8	4.22	≤ 8.4	-6.4 to 4.7	≤ 5.1
MQC	12.8	≤ 5.1	-4.7 to 6.3	≤ 4.5	5.68	≤ 9.0	-4.0 to 3.7	≤ 5.3
HQC	38.9	≤ 6.7	-4.4 to 2.8	≤ 4.2	33.6	≤ 5.5	-3.0 to 2.1	≤ 3.6
ULQC	123	≤ 5.2	-4.1 to 4.1	≤ 4.3	106	≤ 7.8	-8.3 to 8.5	≤ 7.0
Kit 1	3.95	≤ 8.9	-9.2 to 15.0	≤ 7.6	N/A			
Kit 2	158	≤ 4.9	-11.0 to 5.5	≤ 5.8				

Concentrations are whole matrix equivalent (1 in 4 MRD in assay)

Matrix Effects:

Serum	Plasma
Haemolytic LQC: Run 1: 36.6% Run 2: 7.3% Run 3: 19.3% Therefore pass based on 2/3	Haemolytic LQC: Run 1: 32.9% Run 2: 16.0% Run 3: 14.8% Therefore pass based on 2/3
Haemolytic MQC: ≤6.9% RE	Haemolytic MQC: ≤10.8% RE
Lipeamic LQC: ≤12.4% RE	Lipeamic LQC: ≤5.6% RE
Lipeamic MQC: ≤8.0% RE	Lipeamic MQC: ≤3.0% RE

Parallelism:

Serum	Plasma
• 3 of 5 individuals ≤19.7% RE when diluted 16 fold (including MRD) • 5 of 5 individuals ≤15.8%RE when diluted 8 fold (including MRD)	• 3 of 3 individuals ≤14.4%RE when diluted 8 fold (including MRD)

Stability:

	Serum		Plasma	
	%CV	%RE	%CV	%RE
LQC RT	5.4	-3.6	8.2	5.7
MQC RT	3.3	-3.1	1.2	-1.6
LQC 6FT	2.0	1.6	5.2	1.4
MQC 6FT	-6.0	-0.8	0.0	4.2

Kit Lot to Lot Variation v Established P&A Concentrations:

	Serum		Plasma	
	Lot 2 %RE	**Lot 3 %RE**	**Lot 2 %RE**	**Lot 3%RE**
LLOQ	7.3	-2.6*	12.4	-1.3*
LQC	12.7	-2.2	17.3	0.0*
MQC	9.4	-9.4	19.0	-2.6
HQC	6.7	-2.1	4.8	-8.3
ULQC	8.0	0.0	4.8	-4.0

*Extrapolated BLQ result. Lowest point deactivated in run

Singlicate Analysis:

	Serum	Plasma
	%RE to Duplicate QC	**%RE to Duplicate QC**
LQC	-16.5 to -4.3	-7.3 to 10.3
MQC	5.5 to 10.2	-4.0 to 7.6

Multi-plate Stability:

Performed using three replicate plates and assessing duplicate QC samples across the plate expanse. Stability assessed of the mean concentration versus theoretical concentration.

	Serum		Plasma	
	%CV	%RE	%CV	%RE
LQC	3.7	2.4	10.7	4.7
MQC	1.2	-1.6	5.8	7.7
HQC	1.6	-3.6	4.8	3.0

For more information please contact:

Nicola Hickson
nicola.hickson@lgcgroup.com
Mobile: +44 (0)7900 743884

Richard Ward
richard.ward@lgcgroup.com
Mobile: +44 (0)7384 514891

Zafar Alam
zafar.alam@lgcgroup.com
Mobile: +44 (0)7867 670464