血浆样本常温/4℃放置时间对 Simoa NFL 检测值的影响

一.背景及目的

血浆样本中的 NFL 检测作为实验室最常见的样本类型及检测服务需求量最大标志物，此次测试可初步确定在不同操作环境下 NFL 蛋白在血浆样本中的稳定性并可指导后续生产实验中的样本处理操作。

二．实验设计

1.样本准备：

测试所用样本为 EDTA 血浆，便于后期比对数据所有测试条件均使用同一血浆样本完成：3mL 血浆样本涡旋混匀，10000g 离心 5min，取澄清样本每管分装 150μL，-80℃冻存备用；

2.样本处理条件：

处理温度为常温（~23℃）及冷藏（~4℃）两组实验条件，每组设置 7 个保存处理时间，共计 14 个实验条件，其中处理时间为 0 的样本作为正常操作阳性对照，-80℃解冻混匀后与其他测试组样本一同上机检测；

3.上机检测：

使用 Simoa NF-Light Advantage Kit（502625），所有测试样本均一同上机检测，由仪器进行 2 重复 4×稀释，所有标曲点与样本一同 2 重复参与上机检测，试剂盒内参不参与上机检测。

保存温度	序号	保存时间（h）	稀释条件	重复数	上机样本量（μL）	Assay
常温（~23℃）	1	48	仪器 4×	2	110	NF-light (502625) 标曲 2 重复
	2	24	仪器 4×	2	110	
	3	12	仪器 4×	2	110	
	4	8	仪器 4×	2	110	
	5	4	仪器 4×	2	110	
	6	2	仪器 4×	2	110	
	7	0（解冻混匀后直接上机）	仪器 4×	2	110	
冷藏（~4℃）	8	48	仪器 4×	2	110	
	9	24	仪器 4×	2	110	
	10	12	仪器 4×	2	110	
	11	8	仪器 4×	2	110	
	12	4	仪器 4×	2	110	
	13	2	仪器 4×	2	110	
	14	0（解冻混匀后直接上机）	仪器 4×	2	110	

三．实验操作流程：按 Quanterix NFL protocol 及实验室常规技术服务流程

Quanterix™
The Science of Precision Health

Simoa® NF-Light Advantage Kit
Kit Instructions for HD-1/HD-X item 103186

Simoa NF-Light advantage Kit

The Simoa NF-Light assay is a digital immunoassay for the quantitative determination of Neurofilament-Light in serum, plasma and cerebrospinal fluid (CSF). This assay is for research use only and not for use in diagnostic procedures.

Quanterix
Simoa™ NF-light® Reagent Kit
simoa
2°–8°
REF 103186
Quanterix Corp.
Billerica, MA 01821
LBL-184-82 Rev 2.0
Made In USA
For Research Use Only. Not for Use in Diagnostic Procedures.

NF-Light ASSAY DESCRIPTION

Assay format	2-step digital immunoassay
Storage conditions	Reagents (store upright) 2–8°C Reference Calibrators and Controls (single use) ≤–80°C
Total tests per kit	96
Calibration curve fit	4 parameter logistic curve fit, $1/y^2$ weighted
Calibration range	0– ~450 pg/mL
Dynamic range	0– ~1800 pg/mL (plasma and serum) 0— ~45 ng/mL (CSF)
Calibrator volume	334 µL (for 2 replicates in 96 well plate)
Sample and Control dilution factor	Serum/Plasma 4x CSF 100x
Sample aspiration volume	152 µL diluted at bench 38 µL diluted 4x on HD-1/HD-X

The following equations and table describe the volume required for each sample. Samples diluted at the bench should be run using the **Neat** protocol. Plasma or serum samples to be diluted by the instrument should be run using the standard **4x** protocol.

Sampling from 96 well plates:
30 µL *+ (number of replicates × protocol aspiration volume) = µL/well*
Sampling from Nalgene 5 mL tubes:
50 µL *+ (number of replicates × protocol aspiration volume) = µL/tube*

reps	96 well plate		Sample tubes	
	At bench dilution µL	Instrument 4x dilution µL	At bench dilution µL	Instrument 4x dilution µL
1	182	68	202	88
2	334	106	354	126
3	N/A	144	NA	164

NF-Light QUICK GUIDE

Step 1: Allow calibrators and controls to come to room temperature.

Step 2: Import the NF-Light assay definition under Custom Assay; refer to the lot-specific Certificate of Analysis (CoA) for the concentrations of this lot of calibrators and update in the assay definition.

Step 3: Prepare your assay plate – pipette the appropriate volume of calibrators, controls, and samples into the wells.

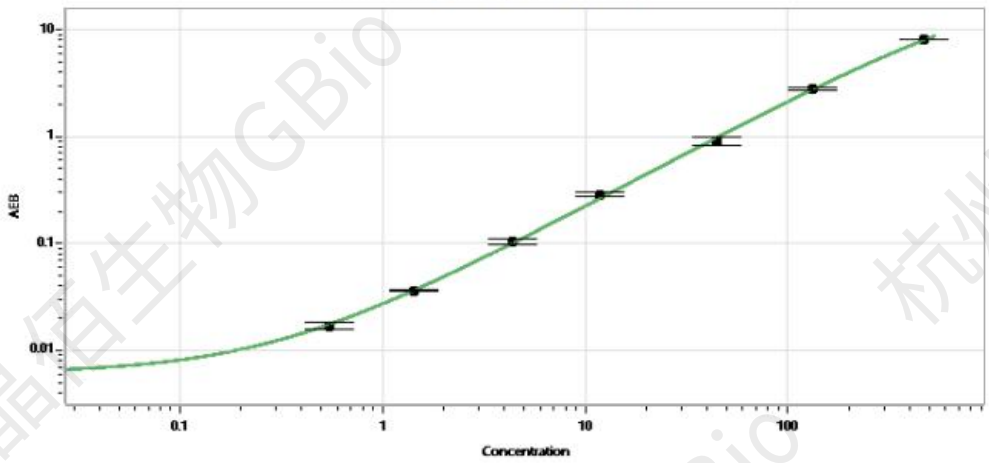
Step 4: Vortex the beads for a minimum of 30 seconds. Remove bottle caps, scan, and load the prepared reagents (Bead Reagent, Detector Reagent, SBG Reagent, Sample Diluent) into the reagent bay and RGP into the sample bay.

Step 5: Set up the assay in Simoa software, select **Neat** or the standard **4x Dilution** protocol for samples and controls, and load the plate on a sample plate rack and into the sample bay.

For information on setting up assay runs, data analysis, and general operating procedures, refer to the HD-1/HD-X user guides and other documents on the Quanterix Customer Portal at http://portal.quanterix.com.

四．实验测试数据

1.标曲数据：



Curve Information	
Weighting Factor	1/y²
R²	0.999895250229148
Fitting Algorithm	4PL
Date Created	6/7/2021 7:48:19 PM
Created by	simoa simoa

Fit Coefficients		Fit Equation
A	0.00611003662740057	$Y(x) = D + \frac{A-D}{1+\left(\frac{x}{C}\right)^B}$
B	1.01589815542027	
C	1225.36185665544	
D	29.1509317100209	

NFL Curve Data

Calibrator	AEB	Mean AEB	AEB CV	Conc. (pg/ml)	Mean Conc.	Repeat
A	0.006	0.006	2.73%	0.000	0.000	Y
	0.006			0.000		
B	0.018	0.017	7.90%	0.544	0.544	Y
	0.016			0.544		
C	0.036	0.036	0.45%	1.430	1.430	Y
	0.036			1.430		
D	0.099	0.104	6.63%	4.410	4.410	Y
	0.108			4.410		
E	0.296	0.289	3.68%	11.800	11.800	Y
	0.281			11.800		
F	0.856	0.916	9.28%	44.500	44.500	Y
	0.976			44.500		
G	2.860	2.783	3.91%	133.000	133.000	Y
	2.707			133.000		
H	8.091	8.045	0.81%	471.000	471.000	Y
	7.999			471.000		
4PL	Approx LOD:	0.0897	pg/ml			
	Approx LLOQ:	0.3580	pg/ml			

Quanterix
The Science of Precision Health

Simoa® NF-light Advantage Kit
Certificate of Analysis

Product Number:	103186
Lot Number:	502625
Expiration:	24-Sep-2021
Platform(s):	HD-1/ HD-X

Component	Part Number	Lot Number
Bead Reagent	102246	026804
Detector Reagent	102248	026904
SBG Reagent	102250	026908
Sample Diluent	102252	026905
Calibrators	102255	030009
Control 1	102256	030201
Control 2	102257	030201
RGP Reagent	103159	N/A[1]

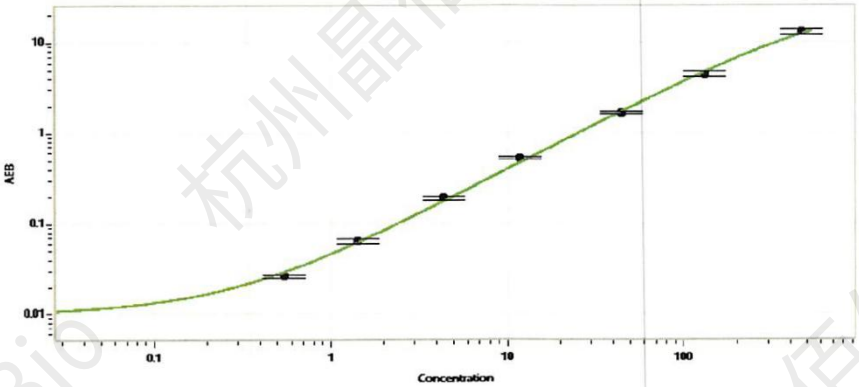
[1] RGP is not Kit Lot Specific

Data below represents results generated on the Simoa HD-X® Analyzer.

Release Materials*			
	Lot	Result (pg/mL)	Mean Range (pg/mL)
Control 1	030201	4.08	2.93 - 5.21
Control 2	030201	159	120 - 203
Panel 1	922601	19.0	14.3 - 21.4
Panel 2	922602	6.67	4.97 - 7.45
Panel 3	922603	40.8	28.8 - 43.2
Panel 4	922604	175	133 - 201

* Ranges shown are generated internally for new lot release only. Customer should generate their own control ranges.

Calibration Curve



Calibrator Levels (pg/mL)	
A	0.000
B	0.544
C	1.43
D	4.41
E	11.8
F	44.5
G	133
H	471

Review/Approval	Junpil Park	QC Supervisor	04Dec2020
	Name	Title	Signature/Date

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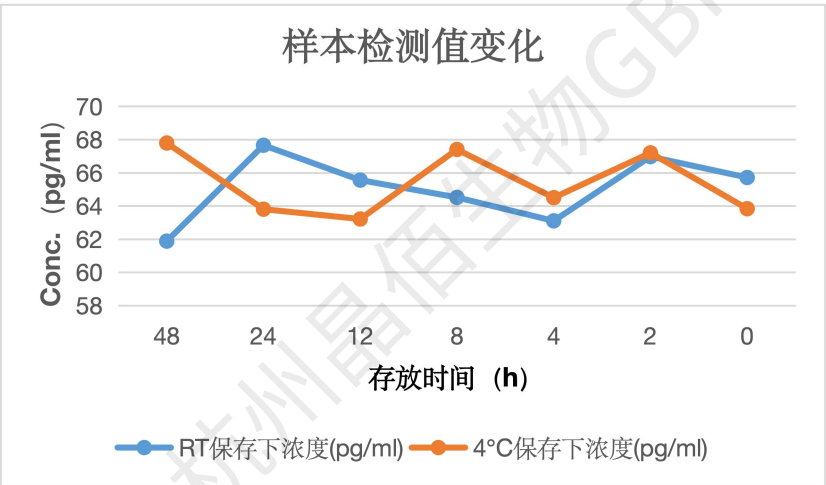
标曲 R^2 及 CV 等质控参数均正常

2.样本数据：

（1）组间组内数据差异

存放时间（h）	RT Conc.(pg/ml)	4℃ Conc. (pg/ml)	CV（%）
48	61.878	67.791	6.4%
24	67.655	63.804	4.1%
12	65.556	63.211	2.6%
8	64.513	67.402	3.1%
4	63.099	64.497	1.5%
2	66.97	67.198	0.2%
0	65.712	63.833	2.1%
平均值	65.055	65.391	/
CV（%）	3.16%	3.03%	/



（2）与对照样本（0 号样本）数据差异

存放时间（h）	CV（RT）	CV（4℃）
48	4.25%	4.25%
24	2.06%	0.03%
12	0.17%	0.69%
8	1.30%	3.85%
4	2.87%	0.73%
2	1.34%	3.63%

五．初步结论

血浆样本内的 NFL 蛋白稳定性较高，在室温/4℃存放 48h 内，对 NFL 蛋白的 Simoa 检测结果无明显影响。