ARGO™ HT System: An Automated, Ultra-Sensitive and High-Throughput Proteomics Platform for the Blood Proteome

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Abstract

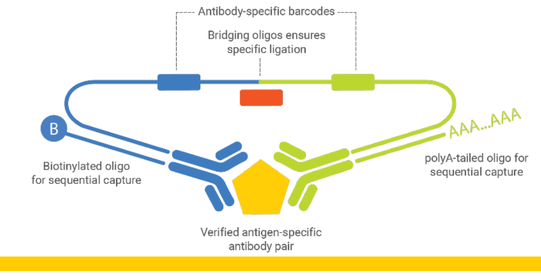
Purpose: Existing proteomic platforms often make tradeoffs between sensitivity and multiplexing capabilities and rely on labor-intensive processes. Here we introduce ARGO HT, an automated high-throughput proteomics platform specifically designed for the NUcleic acid Linked Immuno-Sandwich Assay (NULISA™) technology, a novel immunoassay with attomolar sensitivity and high multiplexing capability. We developed a 250-plex inflammation panel and a 120-plex central nervous system (CNS) disease panel and evaluated their performance on the ARGO HT system.

Methods: The 250-plex inflammation panel contained a wide range of cytokines, chemokines, and other immune-related proteins. It was used to profile the blood of patients with autoimmune diseases (n=21) and COVID-19 at multiple time points (n=46). The 120-plex CNS panel included well-established biomarkers like neurofilament light (NfL) and phosphorylated Tau (p-Tau181, p-Tau217 and p-Tau231) as well as other targets of high relevance to neurodegenerative diseases (NDDs). It was used to analyze plasma (n=38) and cerebrospinal fluid (CSF) (n=29) samples from NDD patients and age-matched controls. Linear model analysis was performed to identify differentially abundant targets between disease and control groups. Coefficient of variation (CV) was calculated for the sample control, and target detectability above the limit of detection (LOD) was calculated for each sample set.

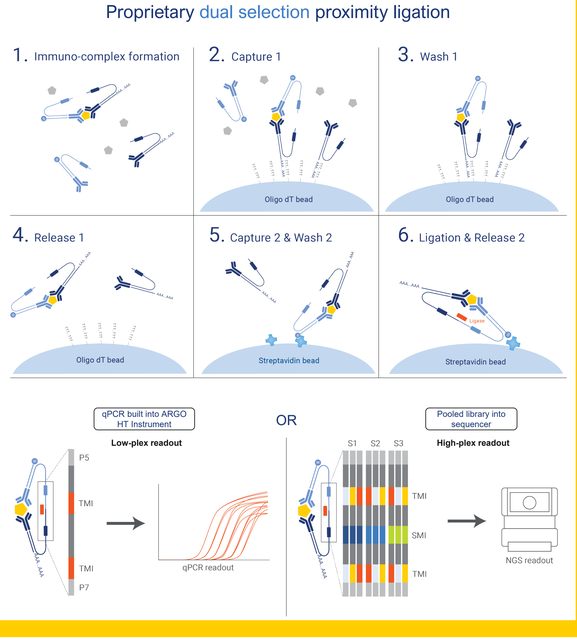
Results: Using 10uL samples, the inflammation and CNS disease panels demonstrated a median CV <10%. Both panels detected over 95% of the targets in plasma, and the CNS panel showed an ~80% target detectability in CSF. Linear regression analysis revealed significant differences in the abundance of both known and novel proteins between disease and control groups.

Conclusion: The benchtop ARGO system fully automates the NULISA technology and demonstrates ultrahigh sensitivity and precision. With the integration of reagents, instrumentation and software ARGO HT democratizes high-throughput proteomic analysis, opening the door to the low-abundance proteome for the discovery of novel biomarkers for early disease detection and therapeutic targets.

NULISA Technology is highly specific with four elements of specificity built into every assay



NULISA workflow drastically improves signal-to-noise ratio and enables multiplexing



NULISA is fully automated on the ARGO HT System for high-throughput analysis of large cohorts or clinical studies

NULISAseq™ Inflammation Panel 250

Most complete coverage of cytokines and chemokines on the market

TARGETS

AGER	CD200	CXCL12	IFNA2	IL-2	LGALS9	SIRPA	TNFSF4
AGRP	CD200R1	CXCL13	IFNB1	IL-20	LIF	SLAMF1	TNFSF8
ANGPT1	CD27	CXCL14	IFNG	IL-22	LIL-RB2	SPP1	TNFSF9
ANGPT2	CD274	CXCL16	IFNL1	IL-23A|EBI3	LTA	TAFA5	TREM1
ANXA1	CD276	CXCL2	IFNL2;IFNL3	IL-23A|IL-12B	LTA|LTB	TEK	TREM2
AREG	CD3E	CXCL3	IFNW1	IL-24	MDK	TGFB1	VCAM1
BDNF	CD4	CXCL5	IKBKG	IL-27|EBI3	MERTK	TGFB3	VEGFA
BMP7	CD40	CXCL6	IL-10	IL-2RA	MICA	THBS2	VEGFC
BST2	CD40LG	CXCL8	IL-10RB	IL-2RB	MICB	THPO	VEGFD
C1QA	CD46	CXCL9	IL-11	IL-32	MIF	TIMP1	VSNL1
CALCA	CD70	EGF	IL-12A|IL-12B	IL-33	MMP1	TIMP2	VSTM1
CCL1	CD80	EPO	IL-12B	IL-34	MMP12	TLR3	WNT16
CCL11	CD83	FASLG	IL-12RB1	IL-36A	MMP3	TNF	WNT7A
CCL13	CD93	FGF19	IL-13	IL-36G	MMP8	TNFRSF11A	
CCL14	CEACAM5	FGF2	IL-13RA2	IL-37	MMP9	TNFRSF11B	
CCL15	CHI3L1	FGF21	IL-15	IL-3RA	MPO	TNFRSF13B	
CCL16	CLEC4A	FGF23	IL-15RA	IL-4	MUC16	TNFRSF13C	
CCL17	CNTF	FLT1	IL-16	IL-4R	NAMPT	TNFRSF14	
CCL19	CRP	FLT3LG	IL-17A	IL-5	NCR1	TNFRSF17	
CCL2	CSF1	FLT4	IL-17A|IL-17F	IL-5RA	NGF	TNFRSF18	
CCL20	CSF1R	FTH1	IL-17B	IL-6	NTF3	TNFRSF1A	
CCL21	CSF2	FURIN	IL-17C	IL-6R	OSM	TNFRSF1B	
CCL22	CSF2RB	GDF15	IL-17F	IL-6ST	PDCD1	TNFRSF21	
CCL23	CSF3	GDF2	IL-17RA	IL-7	PDCD1LG2	TNFRSF4	
CCL24	CSF3R	GFAP	IL-17RB	IL-7R	PDGFA	TNFRSF8	
CCL25	CST7	GRN	IL-18	IL-9	PDGFB	TNFRSF9	
CCL26	CTF1	GZMA	IL-18BP	IRAK4	PGF	TNFSF10	
CCL27	CTLA4	GZMB	IL-18R1	KDR	PTX3	TNFSF11	
CCL28	CTSS	HAVCR1	IL-19	KITLG	S100A12	TNFSF12	
CCL3	CX3CL1	HGF	IL-1B	KLRK1	S100A9	TNFSF13	
CCL4	CXADR	HLA-DRA	IL-1R1	KNG1	SCG2	TNFSF13B	
CCL5	CXCL1	ICAM1	IL-1R2	LAG3	SDC1	TNFSF14	
CCL7	CXCL10	ICOSLG	IL-1RL1	LAMP3	SELE	TNFSF15	
CCL8	CXCL11	IFNA1; IFNA13	IL-1RN	LCN2	SELP	TNFSF18	

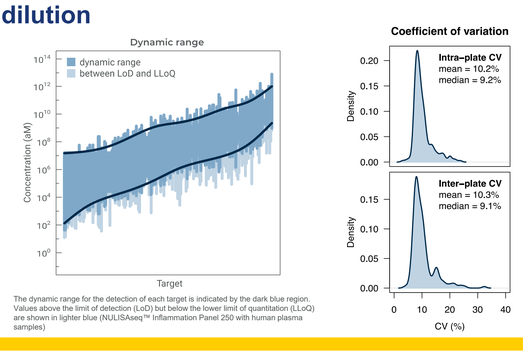
NULISAseq™ CNS Disease Panel 120

Comprehensive panel designed to profile major hallmarks of CNS diseases

TARGETS

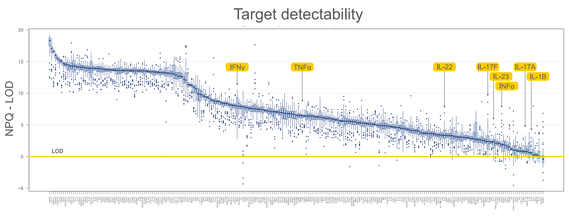
ACHE	CCL4	FOLR1	IL1B	NPTX2	REST	TREM1
AGRN	CD40LG	GDF15	IL2	NPTXR	RUVBL2	TREM2
ANXA5	CD63	GDI1	IL33	NPY	S100A12	UCHL1
Aβ38	CHI3L1	GDNF	IL4	NRGN	S100B	VCAM1
Aβ40	CHIT1	**GFAP**	IL5	Oligo-SNCA	SAA1	VEGFA
Aβ42	CNTN2	GOT1	IL6	PARK7	SFRP1	VEGFD
APOE	CRH	HBA1	IL6R	PDGFRB	SFTPD	VGF
APOE4	CRP	HTT	IL7	PDLIM5	SLIT2	VSNL1
ARSA	CSF2	ICAM1	IL9	PGF	SMOC1	YWHAZ
BACE1	CST3	IFNG	KDR	PGK1	SNAP25	
BASP1	CX3CL1	IGF1	KLK6	pNEFH	SNCA	
CALB2	CXCL1	IGFBP7	**MAPT**	POSTN	SNCB	
CCL11	CXCL10	IL10	MDH1	PRDX6	SOD1	
CCL13	CXCL8	IL12p70	MME	PSEN1	SQSTM1	
CCL17	ENO2	IL13	MSLN	**pTau-181**	TAFA5	
CCL2	FABP3	IL15	NEFH	**pTau-217**	**TARDBP**	
CCL22	FCN2	IL16	**NEFL**	**pTau-231**	TEK	
CCL26	FGF2	IL17A	NGF	pTDP43-409	TIMP3	
CCL3	FLT1	IL18	NPTX1	PTN	TNF	

NULISAseq enables highly reproducible measurements across 12-logs without sample dilution



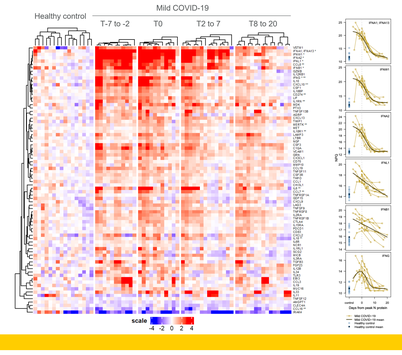
The dynamic range for the detection of each target is indicated by the dark blue region. Values above the limit of detection (LoD) but below the lower limit of quantitation (LLoQ) are shown in lighter blue (NULISAseq™ Inflammation Panel 250 with human plasma samples)

NULISAseq panels have high target detectability in clinical samples



Boxplots showing the distribution of sample NPQ against LOD by target (NULISAseq™ Inflammation Panel 250 with human plasma samples)

Heatmap of SARS-CoV-2- infected patients and healthy controls at different time points



CNS Disease Panel 120 identifies neurodegenerative markers in Alzheimer Disease

AD vs aged-matched healthy plasma (NULISAseq™ CNS Disease Panel 120)

NULISAqpcr™ Single-Plex Assays provide unparalleled sensitivity

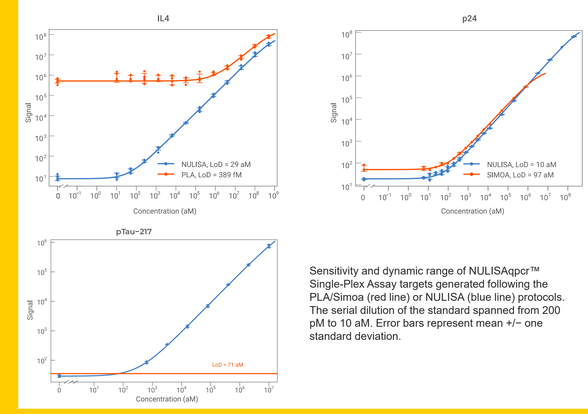
Ultra-high sensitivity measurement of key biomarkers at low to sub fg/mL levels with very small sample input volumes

Inventoried assays

GFAP
IL-4
IL-1B
NfL
pTau-217

Made to order assays

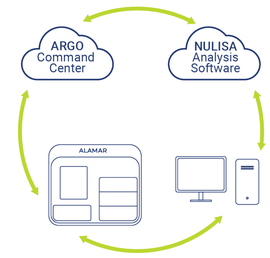
Aβ40	IL6	IL-17F
Aβ42	IL-8	LIF
CCL26	IL-10	pTau-181
IFNγ	TDP-43	IL-12p70
IFNL1	IL-13	TNFα
IL-2	IL-17A/F	tTau
IL-5	IL-17C	



Sensitivity and dynamic range of NULISAqpcr™ Single-Plex Assay targets generated following the PLA/Simoa (red line) or NULISA (blue line) protocols. The serial dilution of the standard spanned from 200 pM to 10 aM. Error bars represent mean +/− one standard deviation.

Alamar Analysis Solutions streamline data analysis

- Perform QC and data normalization for multiplex panels
- Combine data from multiple runs into a single analysis
- Advanced statistical tools for the discovery of novel biological insights
- Generation of publication-ready figures



Materials and Methods

Materials

Healthy and disease human plasma samples were purchased from commercial vendors. The patients in the COVID-19 study were a subset of the mild disease cohort described previously. In brief, samples were collected from January to March 2020 up to six times during the 21 days from inclusion in a study in Germany in the region of North Rhine- Westphalia.

Methods

Multi-plex and Single-plex NULISA assays were performed following the protocol in the reference[1].
NULISAseq and NULISAqpcr data analyses were performed using in house data analysis pipelines.

Conclusions

- NULISA technology is highly specific with four elements of specificity built into every assay.
- NULISA technology effectively reduces assay background noise and enhances the sensitivity, reaching attomolar level of detection.
- The NULISA assays are fully automated on the ARGO™ System, a fully automated, high-throughput precision proteomics platform.
- NULISAseq™ Inflammation Panel 250 and NULISAseq™ CNS Disease Panel 120 provide powerful tools for profiling inflammatory and CNS diseases.
- NULISAqpcr™ Single-Plex Assays provide ultra-high sensitivity at low to sub fg/mL levels.

Reference and contact

1. *Nature Communications* volume 14, Article number: 7238 (2023). https://doi.org/10.1038/s41467-023-42834-x

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