Ultra-sensitive Immune Profiling of Autoimmune Diseases with 200-Plex NULISAseq™ Inflammation Panel

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Abstract

Cytokines and chemokines are critical components of the immune system and play important roles in autoimmune diseases. Comprehensive profiling of proteins in blood can provide deeper insights into the mechanisms underlying this highly complex and heterogeneous group of diseases. However, many of these proteins are present at very low concentrations in plasma, below the limit of detection of current immunoassays. We recently developed a novel automated multiplex immunoassay technology, NULISA™, capable of attomolar-level sensitivity, and a 200-plex inflammation-focused panel targeting 124 cytokines/chemokines and 80 other important inflammation and immune response-related proteins. We analyzed 21 plasma samples from patients with rheumatoid arthritis (n=5), Sjögren's syndrome (n=5), systemic lupus erythematosus (n=5), and ulcerative colitis (n=6), and 79 healthy donor samples using the 200-plex NULISAseq™ Inflammation Panel and the Olink Explore 384 Inflammation assay. Differential expression analysis using linear models identified 114 (56%) and 94 (26%) significant targets at a 5% false discovery rate in the NULISAseq and Olink Explore datasets, respectively. Among the 92 targets shared between these two platforms, 54 and 43 significant targets were identified by NULISAseq and Olink, respectively, with 36 in common. Many of the targets identified only by NULISAseq were low-abundance targets that were poorly detected by the Olink assay (IL4, IL5, IL20, IL17A, IL17F, IL33, and IL2RB) but have important roles in autoimmune diseases. In summary, with improved sensitivity and the most comprehensive inflammatory cytokine/chemokine panel, NULISA promises to be a powerful discovery tool for autoimmune disease research, which may lead to new diagnostic biomarkers and therapeutic targets.

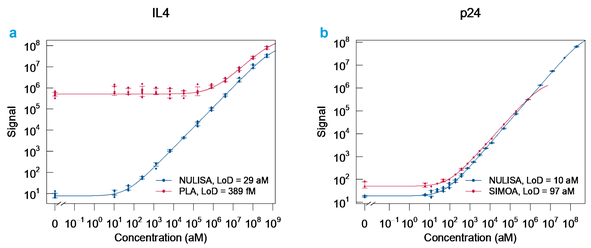
NULISA Technology and Platform

Schematic of the NULISA workflow. 1) Immunocomplex formation; 2) first capture of immunocomplexes to dT beads; 3) bead washing to remove unbound antibodies and sample matrix components; 4) release of immunocomplexes into solution; 5) recapture of immunocomplexes onto streptavidin beads; 6) bead washing and DNA strand ligation to generate reporter DNA; 7a) detection and quantification of reporter DNA levels by qPCR; 7b) quantification of reporter DNA levels by NGS.

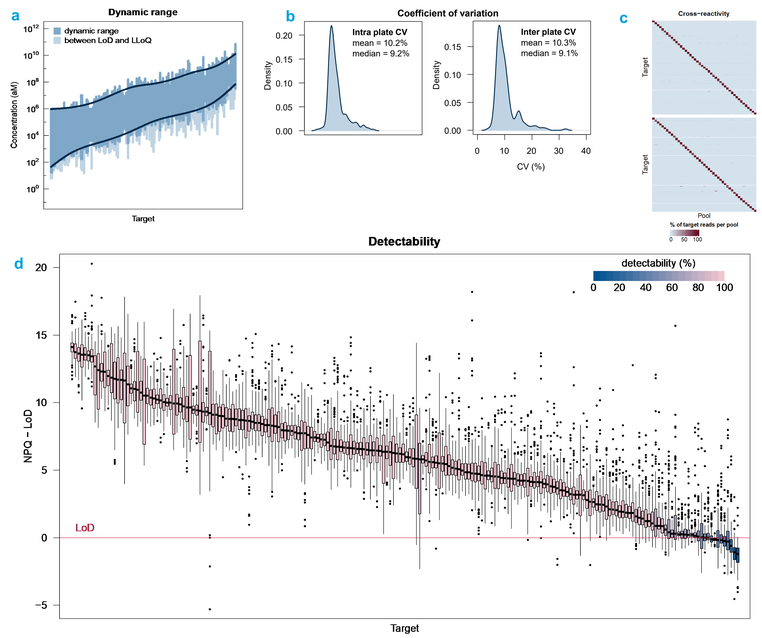
List of selected targets in 200-plex inflammation panel

AGER	AGRP	ANGPT1	C1QA	CCL13	CCL17	CCL20
CCL22	CCL23	CCL24	CCL25	CCL28	CCL3	CCL4
CCL7	CD200	CD200R1	CD276	CD40	CD70	CD83
CLEC4A	CSF1	CSF3	CST7	CXADR	CXCL1	CXCL10
CXCL12	CXCL14	CXCL3	CXCL8	CXCL9	EGF	EPO
FASLG	FGF19	FGF2	FLT3LG	GZMB	HGF	IFNG
IL10	IL10RB	IL11	IL12B	IL12RB1	IL13	IL15RA
IL16	IL17A	IL17C	IL17F	IL17RB	IL18	IL18R1
IL1B	IL1R2	IL1RN	IL20	IL2RB	IL32	IL33
IL3RA	IL4	IL4R	IL5	IL5RA	IL6	IL7
IRAK4	LAMP3	LGALS9	LTBR	MERTK	MMP10	NCR1
OSM	PTX3	TGFB1	TLR3	TNF	TNFRSF11A	TNFRSF13C
TNFRSF14	TNFRSF4	TNFSF10	TNFSF11	TNFSF12	TNFSF13	VEGFD

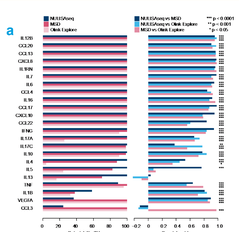
Results

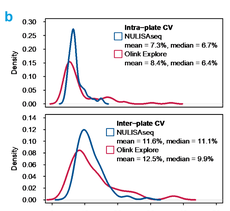


Background suppression in NULISA improved sensitivity. **a.** Standard curves for IL4 detection generated following the traditional PLA or NULISA protocols using the same set of reagents. **b.** Sensitivity and dynamic range comparison between NULISA and SIMOA using the same pair of antibodies for HIV p24.



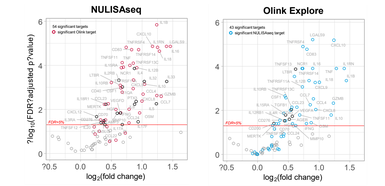
200-plex NULISAseq™ Inflammation Panel performance characterization. a. The dynamic range for each target is indicated by the dark blue region. Values above the limit of detection (LoD) but below the lower limit of quantitation (LLoQ) are shown in lighter blue. Targets are ordered according to the geometric mean of the LLoQ and upper limit of quantitation (ULoQ). Dark blue lines represent generalized additive model (GAM) cubic regression spline fits to the ULoQ and LLoQ. The overall dynamic range for 200-plex NULISAseq spanned 9.6 log10 values. **b**. Density plots of intraplate CV after internal control normalization (**top**) and interplate CV after internal control and intensity normalization (**bottom**). **c**. Cross reactivity. Two sets of 45 random antigen pools containing 4-5 targets each were analyzed with 200-plex NULISAseq. Each cell of the heatmap represents the percent of normalized read counts for that target (rows) occurring in that pool (columns). Targets were ordered according to pool membership such that the cells on the diagonal corresponded to the assigned pools. **d**. Detectability of 204 targets in 151 samples, including 79 from healthy controls and 72 from patients with various diseases. The y-axis represents NULISA Protein Quantification (NPQ) units minus the LoD for the respective target.





Correlation and comparison of NULISAseq with other assay platforms. a. Detectability and interplatform correlation for 23 shared targets in the NULISAseq 200-plex, Olink Explore 384 Inflammation Panel, and MSD V-PLEX Human Cytokine 44-Plex assays. Interplatform correlation was higher for more detectable targets. **b**. Intra- and inter-plate CV distributions for the 92 common targets in the NULISAseq 200-plex and Olink Explore 384 Inflammation Panel.

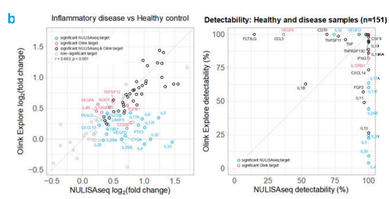
a **Differential expression: Inflammatory disease (n=21) vs Healthy control (n=79)**



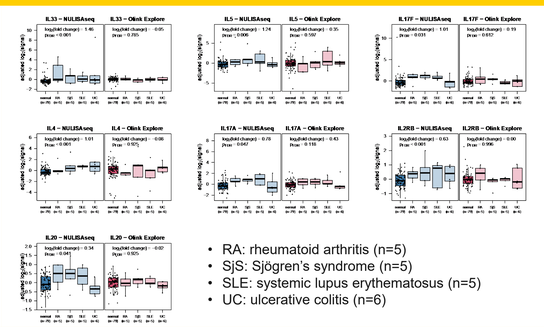
Significant targets

NULISAseq				OlinkExplore
C1QA	IL32	CCL23	GZMB	LTBR	AGER
CCL22	IL33	CCL24	HGF	MERTK	CD200
CXCL12	IL3RA	CCL4	IFNG	MMP10	IL12RB1
FASLG	IL4	CCL7	IL10	NCR1	IL4R
IL17A	IL5	CD276	IL10RB	OSM	TGFB1
IL17F	IL6	CD40	IL12B	TNF	TNFSF13
IL18R1	LAMP3	CD70	IL15RA	TNFRSF11A	VEGFA
IL20	PTX3	CD83	IL16	TNFRSF13C	
IL2RB	VEGFD	CSF1	IL18	TNFRSF14	
		CXCL10	IL1B	TNFRSF4	
		CXCL8	IL1RN	TNFSF11	
		CXCL9	LGALS9	TNFSF12	

b



Differential expression analysis comparing 21 samples from patients with inflammatory disease and 79 samples from healthy controls using the 92 shared targets between NULISAseq and Olink Explore. **a.** Volcano plot of log10(FDR-adjusted p-value) versus log2(fold change) levels detected using NULISAseq (**left**) and Olink Explore (**middle**). **Right**, Venn diagram showing the overlap of significantly different targets between the two assays. **b**. Correlation of estimated log2-fold changes between NULISAseq and Olink Explore. Targets detected by one platform but not the other are highlighted in blue (detected by NULISAseq only) or red (detected by Olink only). Targets in black were detected by both panels, and targets in grey were not significant for either panel.



- RA: rheumatoid arthritis (n=5)
- SjS: Sjögren's syndrome (n=5)
- SLE: systemic lupus erythematosus (n=5)
- UC: ulcerative colitis (n=6)

Select low-abundance targets associated with autoimmune diseases detected by NULISAseq but not by Olink Explore. Boxplots show the distributions of residuals (after adjusting for age, sex, and plate) for healthy controls versus inflammatory disease subgroups. FDR-adjusted p values were obtained based on the disease status (inflammatory disease, n=21, versus healthy control, n=79) coefficient from linear models (adjusted for age, sex, and plate) used in the differential expression analysis of 92 shared NULISAseq and Olink targets.

Conclusions

- NULISA achieves attomolar sensitivity by incorporating a unique background suppression mechanism to reduce assay background to a minimum
- NULISAseq enables high multiplexing and sample throughput while maintaining single attomolar sensitivity
- NULISA's ultrahigh sensitivity enabled detection low-abundance targets that were poorly detected by the Olink assay (e.g., IL4, IL5, IL20, IL17A, IL17F, IL33, and IL2RB) but have important roles in autoimmune diseases.
- NULISA provides a powerful new tool for both biomarker discovery and validation, which should facilitate seamless translational research.

Materials and Methods

Patient Samples
Human plasma samples were purchased from BioIVT (Westbury, NY).

Methods
21 plasma samples from patients with rheumatoid arthritis (n=5), Sjögren's syndrome (n=5), systemic lupus erythematosus (n=5), and ulcerative colitis (n=6), and 79 healthy donor samples were analyzed by using the 200-plex NULISAseq™ Inflammation Panel and the Olink Explore 384 Inflammation Panel. The NULISA assay was performed at Alamar Biosciences (Fremont, CA), and the Olink PEA assay was performed by Fulgent Genetics (Temple City, CA) and the High Throughput Biomarker Core of Vanderbilt University (Nashville, TN).

References:

Feng, W., Beer, J.C., Hao, Q. *et al.* **NULISA: a proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing**. *Nat Commun* **14**, 7238 (2023). https://doi.org/10.1038/s41467-023-42834-x

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