NULISA: Fully automated ultra-sensitive immunoassay platform for profiling fluid-based protein biomarkers

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WACD
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**Mission:** Power precision proteomics to enable the earliest detection of disease

- **NULISA™** - ultra-sensitive Attomolar ($10^{-18}$M) NGS enabled digital immunoassay platform for protein analysis from 1 – 1000’s of plex

- **Attobody™**: bi-paratopic antibodies with picomolar affinity

- **ARGO™** - Fully automated solutions to perform assay across a range of multiplex levels and applications from discovery to the clinic

Automated solutions for **ultra-sensitive** protein analysis across a range of **multiplex** level to detect **critical biomarkers** from discovery to the clinic.
NULISA™ platform enables greater sensitivity and precision in high-plex proteomics

- Best-in-class Attomolar (fg/ml) sensitivity increases the depth of measurable proteins
- Enabling detection of known biomarkers at lower levels
- Enabling discovery of novel biomarkers that are currently undetectable
- Enabling clearer definition of health baseline biomarker levels
**NULISA™: the most sensitive immunoassay liquid biopsy platform**

- **Unprecedented sensitivity**
  - Attomolar (fg/mL) level of detection
  - Quantification of low abundance proteins
  - Enables discovery of critical biomarkers

- **Widest dynamic range**
  - >12 logs dynamic range
  - Quantification across biological range of protein levels
  - In one reaction w/o dilution

- **Flexible, multiplex chemistry**
  - Single target or multiplex
  - 100s to 1000s of targets in a single experiment
  - Broad sample compatibility

- **Fully automated**
  - Ease of use
  - Minimal hands-on time
  - High reproducibility
Precision proteomics at the **push of a button**

Full solution of instrument, reagent kits, and software

**ARGO™ HT System**

**Consumables**
Simple, robust, reproducible

**Instrument**
ARGO all-in-one instrument w/ onboard qPCR

**Software**
From samples to insights
Scalability and Flexibility All-in-one instrument

**Discovery**
100s of markers with high sensitivity for focused discovery within known biology

**Translation**
Multiplex flexibility to validate potential clinical targets with high sensitivity and precision

**Clinical Biomarkers**
Single to low-plex qPCR-based assay kits for biomarkers and future diagnostics
NULISA Technology and how it works?

- Bridging oligos ensure specific ligation.
- Antibody-specific barcodes.
- PolyA-tailed oligo for sequential capture.
- Biotinylated oligo for sequential capture.
- Verified antigen-specific antibody pair.
**NULISA Workflow**

- **Step 1:** in solution antibody – antigen immune complex formation

- **Step 2:** 1st bead-based capture – polyA-linked capture antibody on poly-dT beads
NULISA Workflow

• **Step 3:** wash – removal of all free detection antibodies, analytes and matrix; retention of specific analyte bound by capture antibody

• **Step 4:** release of capture antibody/analyte complex
• **Step 5:** 2nd bead-based capture via biotinylated oligo binding to streptavidin

• **Step 6:** wash – removal of all antibodies and residual matrix not complexed with analyte and 2nd detection antibody
NULISA Workflow

- **Step 7:** ligation of specific bar-coded oligos

- **Step 8:** wash, then qPCR or NGS
NULISA achieves the highest sensitivity with the same antibodies using proprietary assay background suppression.

IL-4 and HIV p24 NULISA using the same antibodies: NULISA vs PLA and SIMOA

NULISA lowers the limit of detection and increases the quantifiable dynamic range with less sample.

Source: https://www.biorxiv.org/content/10.1101/2023.04.09.536130v1
Routine and reliable attomolar (fg/mL) LOD for single-plex NULISA

- **IL-4 NULISA**
  - LOD = 1.3 fg/ml

- **IL-6 NULISA**
  - LOD = 3.1 fg/ml

- **IL-10 NULISA**
  - LOD = 1.0 fg/ml

- **LIF NULISA**
  - LOD = 1.3 fg/ml

- **IL-13 NULISA**
  - LOD = 0.2 fg/ml

- **IFNγ NULISA**
  - LOD = 12.5 fg/ml
NULISA “sample-in-data-out” platform enables high precision analysis of single and multiplexed analytes

Concordance, high sensitivity and wide dynamic range for single-plex qPCR and multi-plex NGS NULISA readouts

Cytokine level (fM)

Five cytokines from LPS stimulated whole blood samples from 3 healthy donors were assessed by NULISAseq or NULISA using 1.33 µL of plasma per assay

• Donor 1
• Donor 2
• Donor 3
**NULISAs**seq™ Inflammation Panel

Most complete coverage of cytokines and chemokines on the market

- **250+ biomarkers in 10µL**
- **Attomolar sensitivity** (10 fg/mL)
- **~12 logs dynamic range without dilution**
- **Highly reproducible** CV <10%
- **Customizable** Subset panels and panel+

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<th>TARGETS</th>
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NULISA: a novel proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing

https://www.biorxiv.org/content/10.1101/2023.04.09.536130v2

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NULISA has tighter CV% versus Olink

200-plex NULISA
Intra- and inter-plate median CV within 10%

Intra-plate CV for NULISA and Olink for 92 shared targets in 159 plasma samples

<table>
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<tr>
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<th>CV% Plate 1 &amp; 2 Average</th>
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<tr>
<td>NULISAseq</td>
<td>7.3 (2.4) / 6.6 [4.1, 15.3]</td>
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<tr>
<td>Olink Explore</td>
<td>9.4 (8.0) / 6.5 [2.6, 54.4]</td>
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NULISA shows greater detection of differential expression of key inflammatory markers versus Olink.

NULISA identified significant differences

C1QA CCL3 CSF1 IL15RA IL17F IL2RB II32 II33 IL4 IL5 IL6 LTBR OSM PTX3 TNFRSF13C VEGFD

CCL3

CCL24 IL18 CCL4 IL1B CCL8 IL1RN CD83 LGALS9 CXCL10 MMP10 CXCL8 NCR1 CCXCL9 TNF GZMB TNFRSF11A IL10 TNFRSF14 IL12B TNFRSF4 IL16

NULISA & Olink identify significant differences

Attributable to poor antibody performance

NULISA vs Olink fold-differences:
Inflammatory disease and healthy donor plasma

NULISA & Olink identify significant differences

Inflammation vs. Healthy

R = 0.66

Feng, et al., (2023) BioRxiv

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Detectability of NULISA Inflammation Panel in plasma

99.6% of targets were detectable in at least 50% of samples
Stanford Human Immune Monitoring Core Adopts NULISA and ARGO on performance and workflow automation

- NIH IMPACC Study of COVID-19 patients
- Three-way comparison between NULISAsseq Inflammation Panel, Olink and Luminex.
- NULISAsseq showed the highest detectability, which translated to biological insights not obtained from the other two platforms
- Feedback from this customer: impressed with high sensitivity and fully automated workflow
- Signed up as first beta customer.

“NULISA platform showed a higher sensitivity for a fair number of analytes and beyond the system's sensitivity, the fact that it comes packaged as an automated platform is attractive, especially given that PEA has historically been a complicated process.”
NULISAseq™ CNS Disease Panel – Available Q1 2024

Targets (120 Plex)

<table>
<thead>
<tr>
<th>Amyloid Beta 38</th>
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CNS Disease Panel: Detecting key differences in plasma between age-matched and Alzheimer’s Disease samples

Significantly up-regulated markers include:
- Phospho-Tau181
- Phospho-Tau217
- Phospho-Tau231

Significantly down-regulated markers include:
- S100A12
- ENO2
ARGO™ HT Instrument
Precision Proteomics at the Push of a Button

- Simple, automated workflow
  <30 min hands-on time

- Integrated data analysis
  Cloud-based analytics

- Minimal sample input
  10 – 20 µL

- Single plex results <8 hrs
  On-board qPCR analysis

- Multiplex workflow
  Outputs NGS-ready libraries

- High throughput
  Up to 288 samples in 3 x 96-well plates per day
Proteomics at the push of a button
Enabling global access of proteomic analysis from sample to data

Fully Automated Workflow with ARGO™ HT

1 LOAD
Simple, robust and reproducible
< 30 min hands-on time

2 RUN
High throughput sample-to-results
< 8 hours

3 NGS
NextSeq2K (500m reads/run)
~ 8 hours

4 ANALYZE
Cloud-based analytics

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ARGO™ System offers 3x faster and 20x simpler workflow solution
Thank you for your attention

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For more information
Visit www.alamarbio.com

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