Next Generation Protein Detection

Oak Ridge Conference

Introducing Longitudinal Assay Screening (LAS)

April 19, 2013

inanovate
Opening doors to a smaller world

Introduction to Inanovate

Location	Research Triangle, NC; Boston, MA
Technology	**Longitudinal Assay Screening**
Associated Product	Bio-ID 400
Application Focus	Detection, measurement and monitoring of multiple proteins
Under Development	• Automated near-care platform (Bio-ID Dx) • Diagnostic biomarker panels for prostate cancer, ovarian cancer and sepsis.
Selected Partnerships & Collaborations (public)	MD Anderson, Harvard Medical School, Thermo Fisher, Dana Faber Cancer Center, Manchester Hospital (NHS Trust)

Protein Multiplexing: The Problems

Accurately detecting and measuring single, let alone **multiple proteins** is a significant scientific and technical achievement.

The development of Longitudinal Assay Screening (LAS) was driven by three limitations with existing technologies

- **Limited Detection Range**
- **Limited Biological Relevance**
- **Limited Data Accuracy**

Protein Multiplexing: The Problems

Image borrowed from Journal of Proteome Research - 2011, 10, 5-16 - Silvia Surinova et al

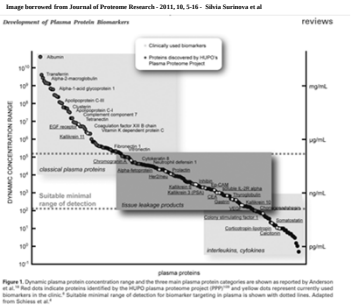


Figure 1. Dynamic plasma protein concentration range and the three main plasma protein categories are shown as reported by Anderson et al.[10] Red dots indicate proteins identified by the HUPO plasma proteome project (PPP)[11] and yellow dots represent currently used biomarkers in the clinic.[9] Suitable minimal range of detection for biomarker targeting in plasma is shown with dotted lines. Adapted from Schiess et al.[9]

Limited Detection Range

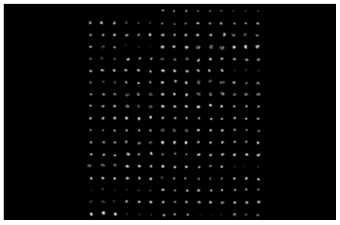
Auto-immune diseases, e.g. rheumatoid arthritis.

Inflammatory markers: Impact cardiovascular disease to cancer.

Limited Biological Relevance

Protein Multiplexing: The Problems

Which signal is 'real' and which is non-specific background?



Limited Data Accuracy

Increases time and cost of biomarker discovery and validation programs, and introduces 'false positive errors' into clinical applications.

The Inanovate Solution: Longitudinal Assay Screening (LAS)

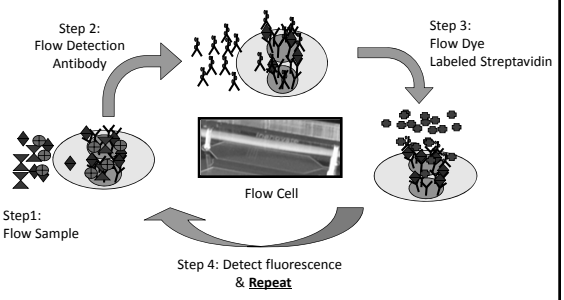
Inanovate has overcome the problems of protein multiplexing through the development of a new category of protein screening technology:
Longitudinal Assay Screening (LAS)

The Bio-ID

A new solution to protein detection

Inanovate has recently completed testing and demonstration of the world's first protein detection platform to integrate LAS technology: **The Bio-ID 400**.

What is LAS?



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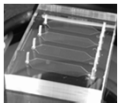
The Bio-ID: First platform to integrate LAS technology

The Bio-ID Detector

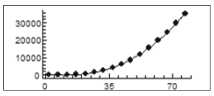
The hardware that detects, processes and analyzes the assays on the Bio-ID's disposable 'Chips'

The Bio-ID Disposable 'Chip'



A fluidic cartridge that houses the assays and facilitates compatibility with the Detector.

Real-time Biomarker Measurement



Iterative flow of sample and detection antibody across the surface of the protein array

Real-time detection, kinetic signatures, increased confidence, walkaway automation!

VIDEO

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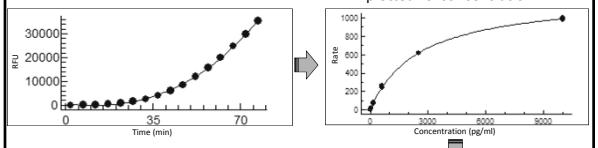
Analyzing LAS data

The Bio-ID captures time course fluorescent data

Rates are calculated and plotted vs. concentration



The rate based binding curve is used to quantify unknowns

IL6	
Vmax	1255.10
n	0.974
Km	2588.39

HQD	53,233
LDD	0.85
LQD	1.70

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The Benefits

- Rate based analysis enables accurate quantitation across over a 7log range in a single multiplex. Eliminates need for serial dilutions, making multiplexing faster, cheaper and helping preserve precious samples.
- Large detection range, LAS allows users to run virtually any assay of interest in one test, enabling the development of truly biologically relevant multiplexes.
- Analysis of real-time kinetic data improves identification and discrimination of background and non-specific signals, delivering more accurate quantitation at low analyte concentrations.

Limited Detection Range

Limited Biological Relevance

Limited Data Accuracy

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Demonstration data:
Improving detection range & biological relevance

To illustrate the Bio-ID's capacity to quantitate very different protein concentrations in a single run, known low abundance proteins (IL-6 and IL-1b) were analyzed in the same 3-plex assay as a known high abundance protein (CRP)

Assay	LDD	HQD	Inter-run %CV (cross instrument)
IL-6	0.85	53,000	3.0
IL-1b	1.26	21,000	6.0
CRP	21.89	1,361,000	5.3

The Bio-ID 400 is an early stage technology. Significant further improvement is likely as the system and assay are further optimized through the coming months.

Large detection range = resource savings + biologically relevant assays

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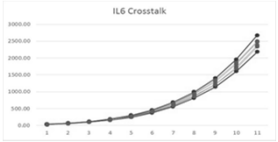
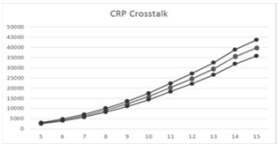
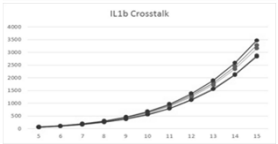
Demonstration data:
Improving detection range & biological relevance

Despite CRP being present at up to 1,000,000X the concentration of IL-6 and IL-1b, there is **no significant cross-talk from CRP to the IL-6 and IL-1b assays.**







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Demonstration data:
Improving detection range & biological relevance

Assay	Level	% Recovery	
		Simulated Matrix	Human Serum
IL-6	High	93.2	108.8
	Mid	75.4	72.2
	Low	96.9	117.3
IL-1b	High	97.6	102.6
	Mid	79.9	99.2
	Low	94.7	106.6
CRP	High	129.6	80.2
	Mid	94.4	90.2
	Low	88.9	73.4

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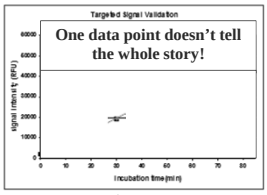
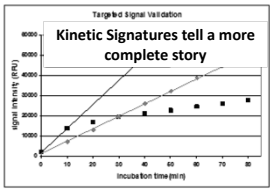
Demonstration data:
Improving data accuracy



Orange data from known 30K pg/mL IL1-b standard. Blue from printed IL1-b detector Ab that reacts with streptavidin (simulated non-targeted signal).



Assay Specific Signatures:
- Specific vs. non specific binding
- Curve shape
- Vmax
- Assay time-course

Summary & Conclusions:
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Limited Biological Relevance

- Analysis of real-time kinetic data improves identification and discrimination of background and non-specific signals, delivering more accurate quantitation at low analyte concentrations.

Limited Data Accuracy

Summary & Conclusions:
Demo 3-plex data from the Bio-ID 400

Assay	LDD	HQD	Inter-run %CV	% Recovery
IL-6	0.85	53,000	3.0	96.7
IL-1b	1.26	21,000	6.0	97.6
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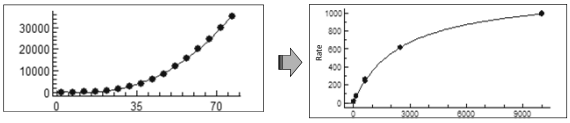
Large detection range = resource savings + biologically relevant assays



Kinetic analysis = high quality data, non-targeted discrimination & improved accuracy

Thank you for your attention

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