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

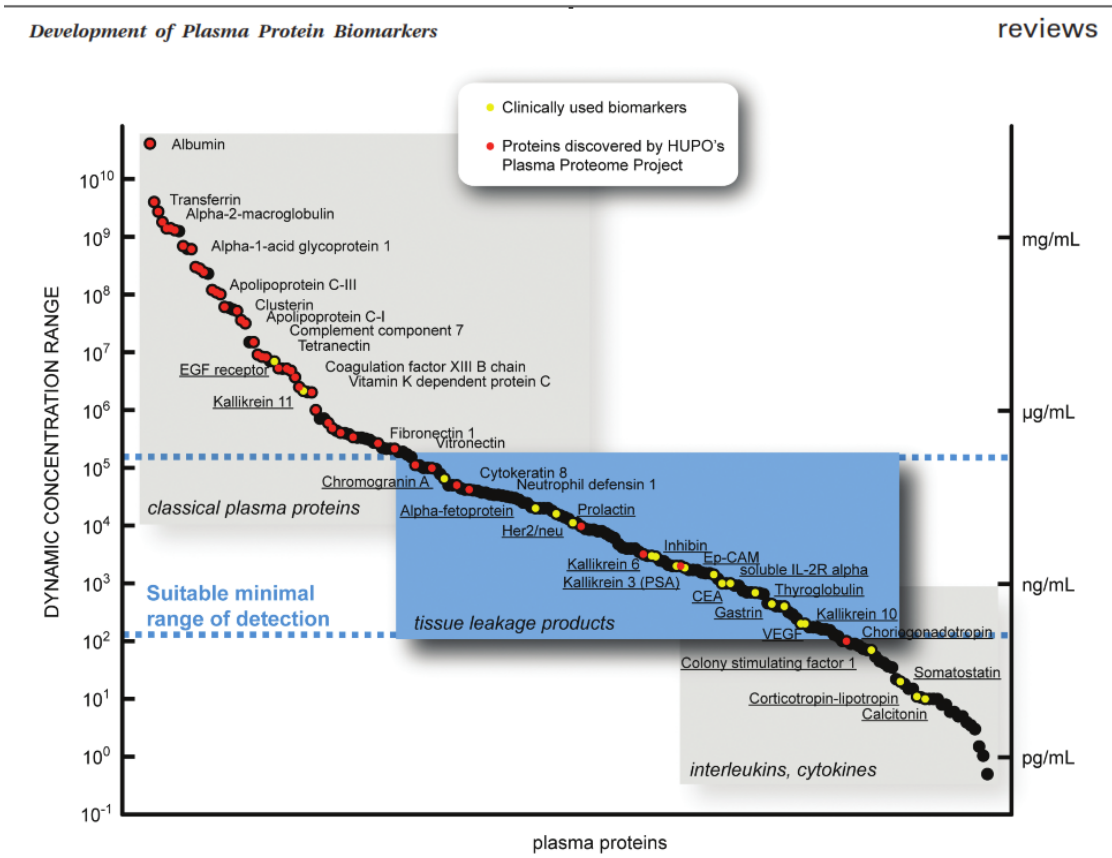


Figure 1. Dynamic plasma protein concentration range and the three main plasma protein categories are shown as reported by Anderson et al.[18] Red dots indicate proteins identified by the HUPO plasma proteome project (PPP)[109] and yellow dots represent currently used biomarkers in the clinic.[6] Suitable minimal range of detection for biomarker targeting in plasma is shown with dotted lines. Adapted from Schiess et al.[4]

Limited Detection Range

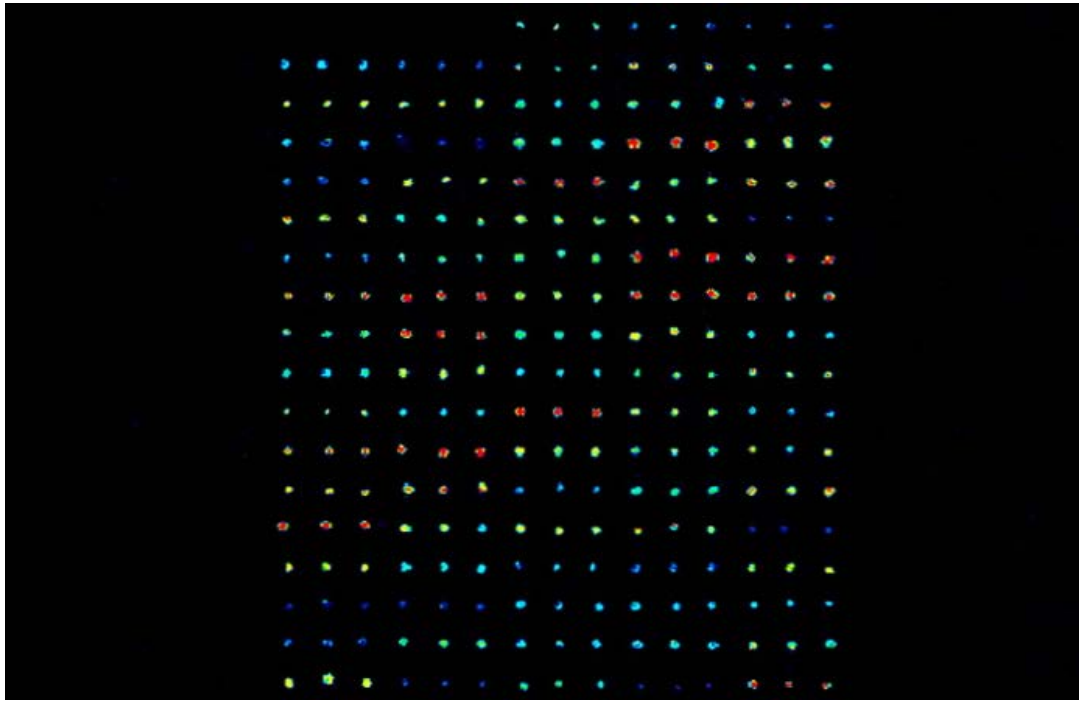
Auto-immune diseases, e.g. rheumatoid arthritis.

Inflammatory markers: Impact cardiovascular disease to cancer.

Limited Biological Relevance

Protein Multiplexing: The Problems

Which signal is ‘real’ and which is non-specific background?



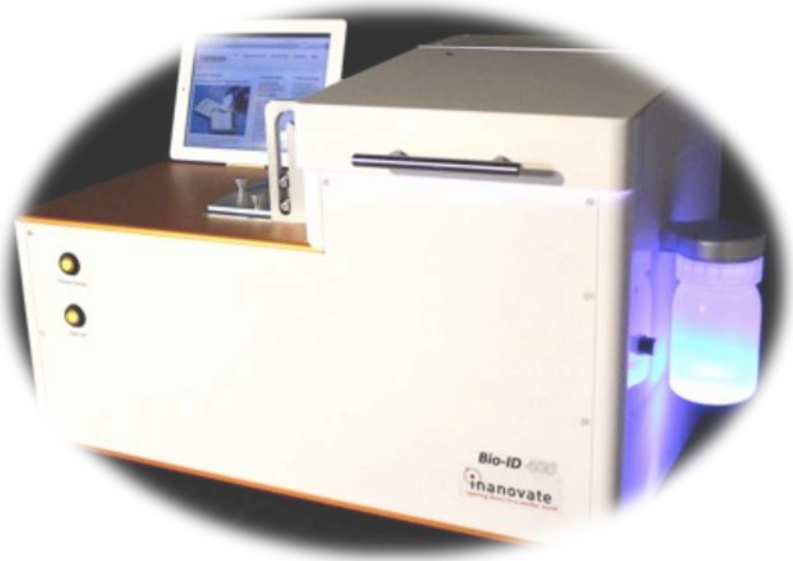
Limited Data Accuracy

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)

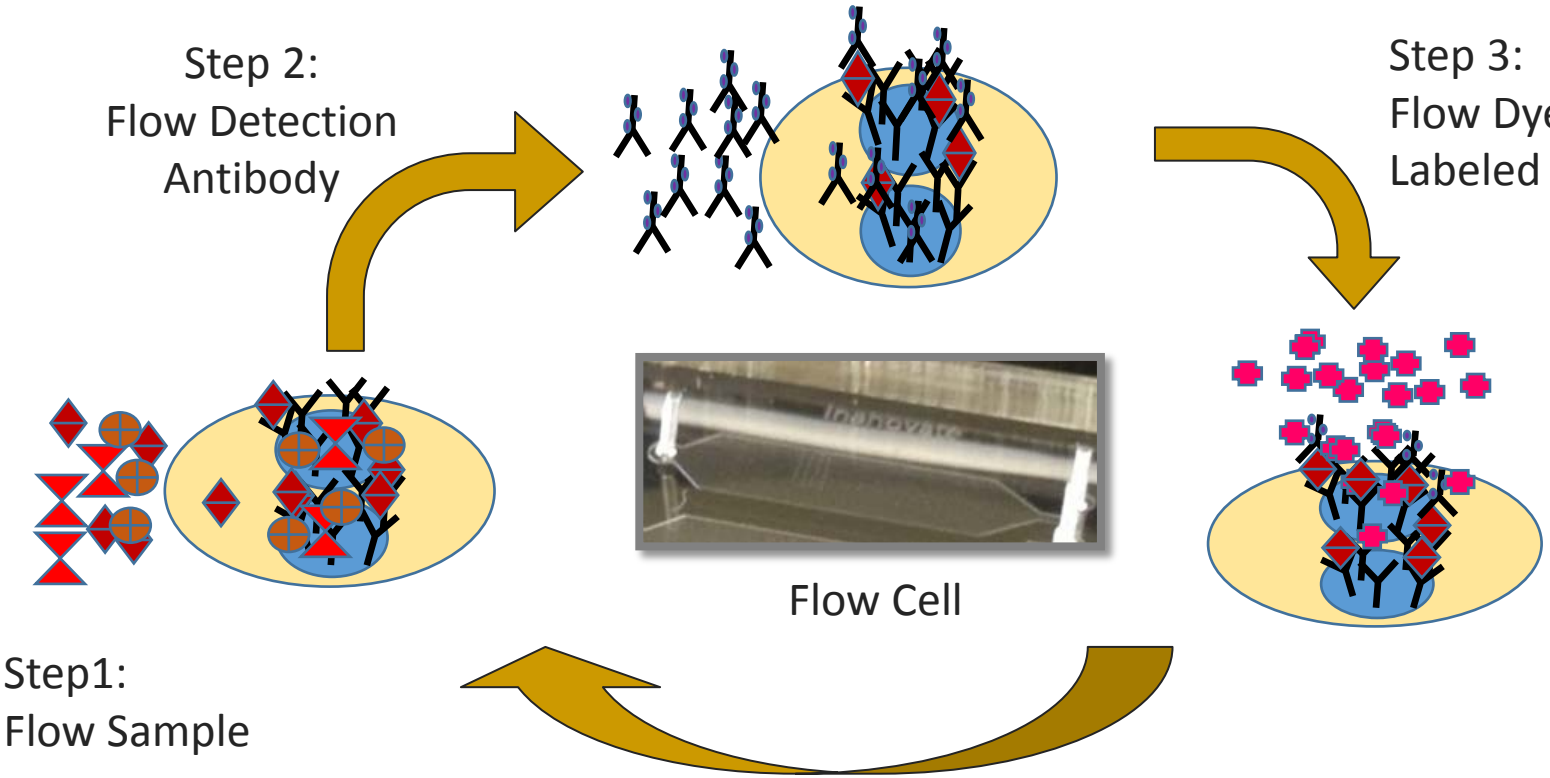
The Bio-ID



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?

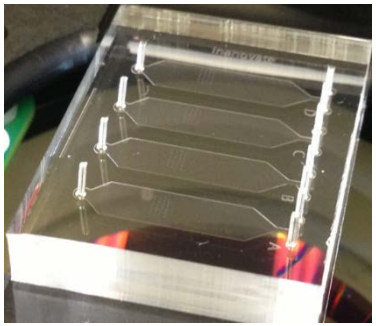

Step 2:
Flow Detection
Antibody
Step 3:
Flow Dye
Labeled Streptavidin
Flow Cell
Step1:
Flow Sample
Step 4: Detect fluorescence
& **Repeat**

The Bio-ID: First platform to integrate LAS technology

The Bio-ID Detector

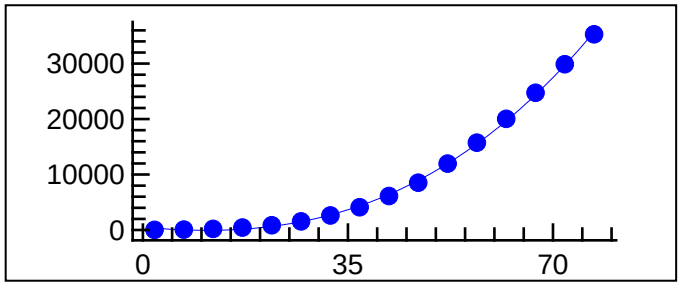
The hardware that detects, processes and analyzes the assays on the Bio-ID's disposable 'Chips'

The Bio-ID Disposable 'Chip'



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

Real-time Biomarker Measurement



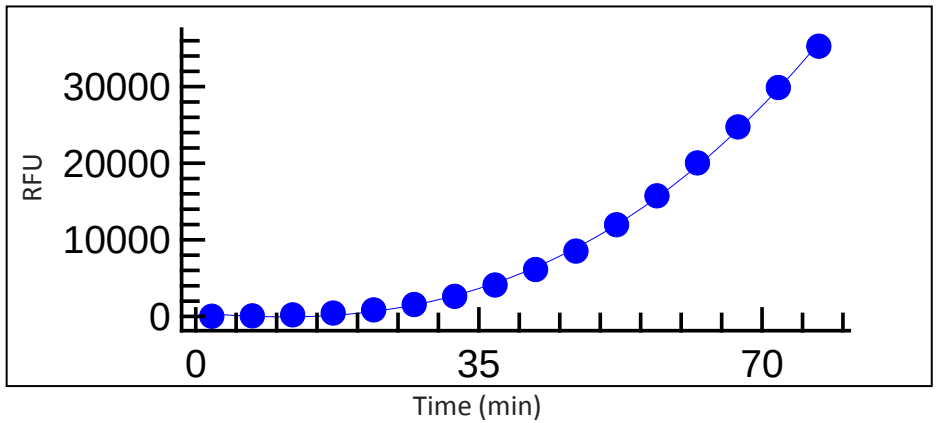
Iterative flow of sample and detection antibody across the surface of the protein array

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

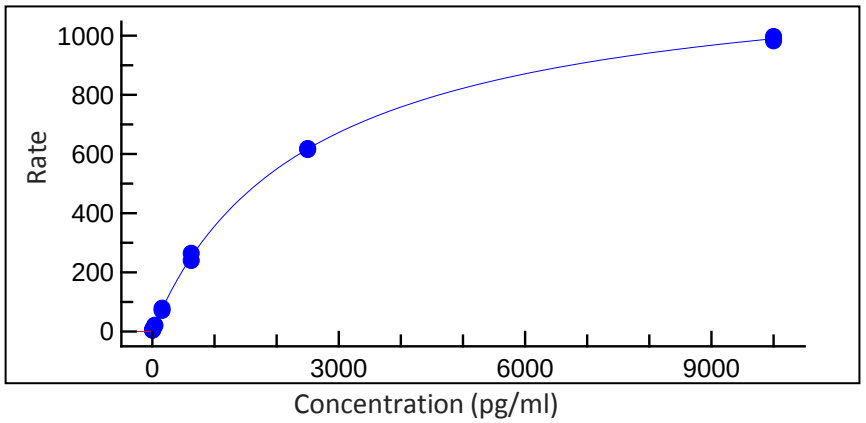
VIDEO

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

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

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)

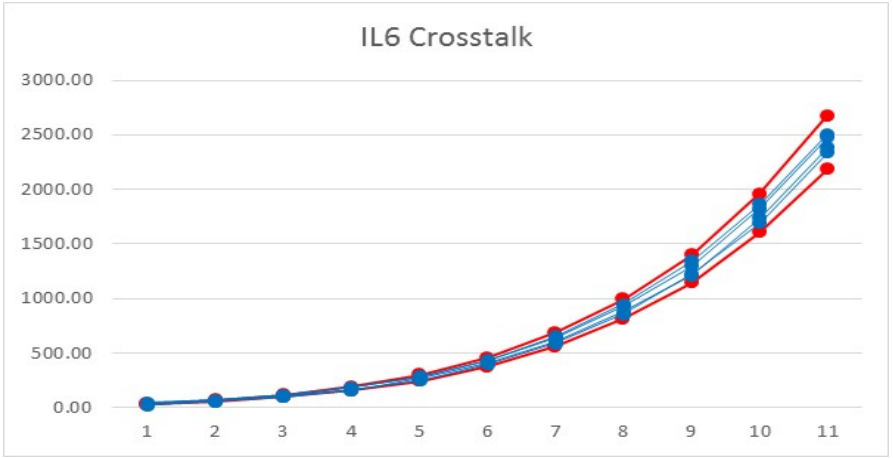
Assay	LDD	HQD	Inter-run %CV (cross instrument)
IL-6	0.85	53,000	3.0
IL-1b	1.26	21,000	6.0
CRP	21.89	1,361,000	5.3

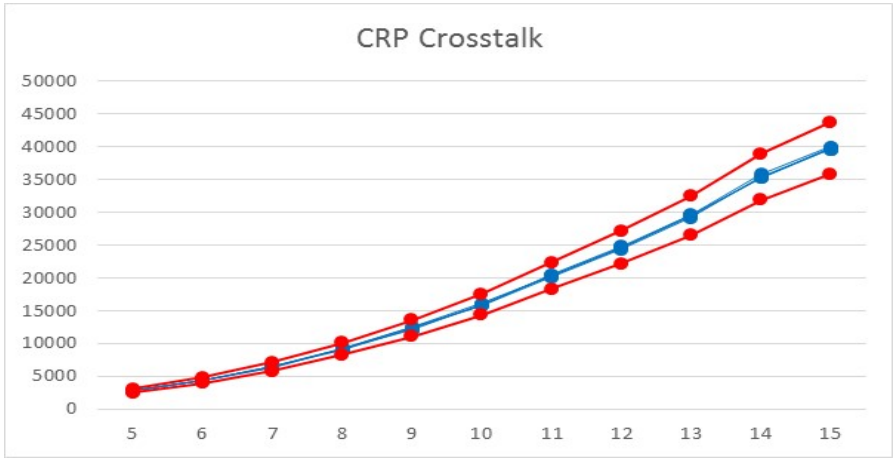
The Bio-ID 400 is an early stage technology. Significant further improvement is likely as the system and assay are further optimized through the coming months.

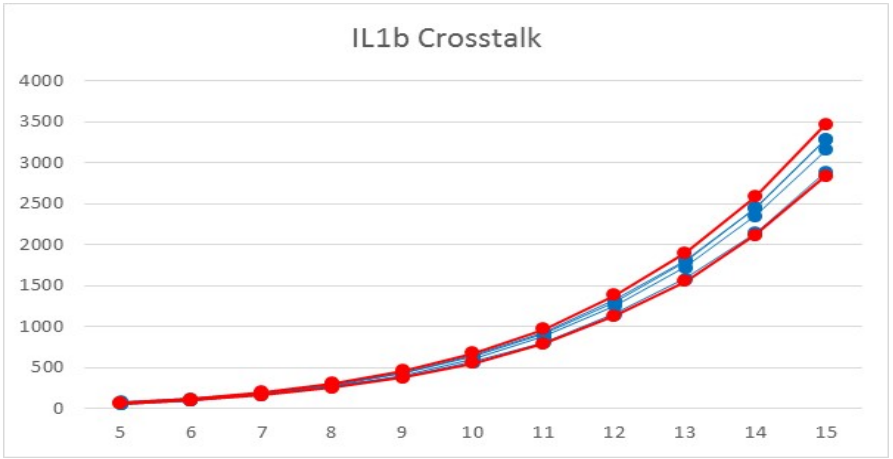
Large detection range = resource savings + biologically relevant assays

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.**







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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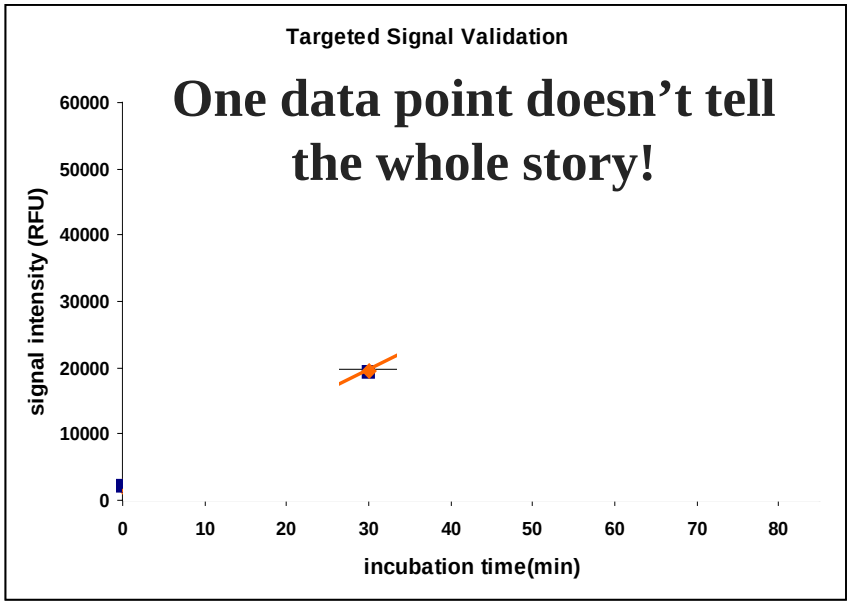
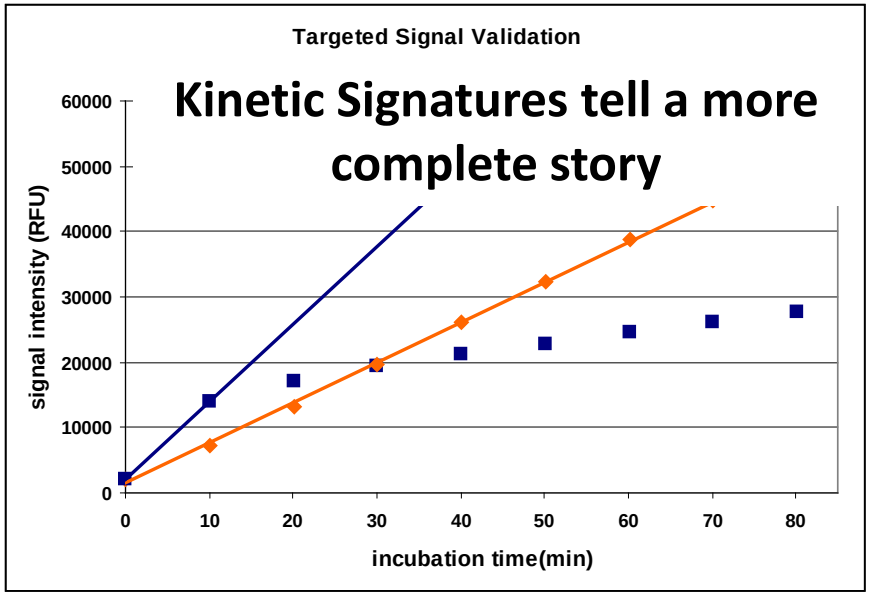
Demonstration data:
Improving data accuracy



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

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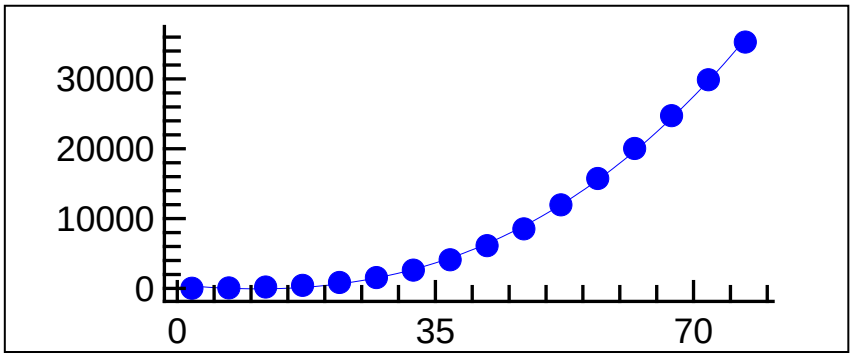
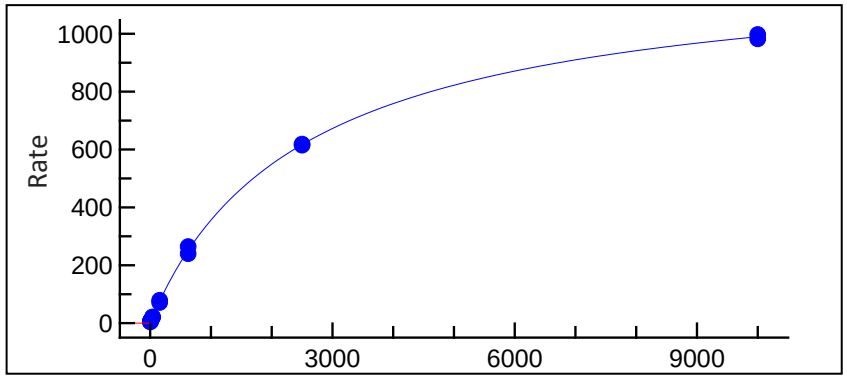
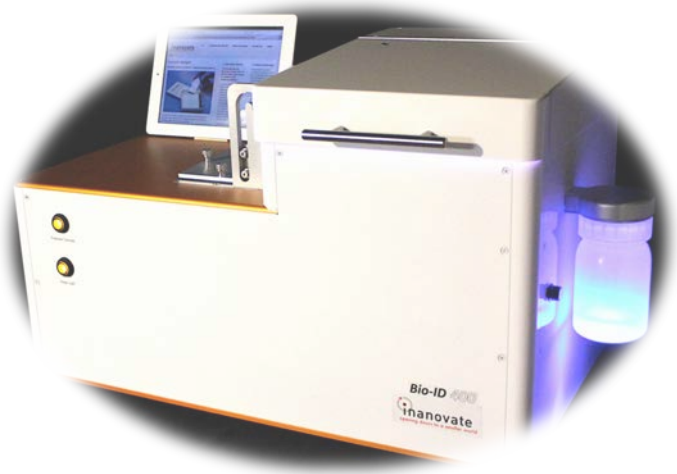




Kinetic analysis = high quality data, non-targeted discrimination & improved accuracy

Thank you for your attention



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