Single Molecule Array (Simoa) technology for ultrasensitive, multiplexed detection of proteins and nucleic acids
Corporate Overview

- Founded 2007 by David Walt and Nicholas Naclerio
- Exclusive licensee of patent portfolio developed by David Walt at Tufts University
- ~ 50 employees, 15 Ph.D’s
- Expanded to state-of-the-art 20,000 sq. ft. facility April 2012 in Lexington, MA
- Venture-backed by leading life science investors and strategic partners
- Cumulative $47M Raised in Series A, B, C

Board of Directors

- Martin Madaus, Ph.D. (Executive Chairman)
- John Connolly (Bain Capital Ventures)
- David Walt, Ph.D. (Tufts University, Illumina)
- Keith Crandell (ARCH Venture Partners)
- Doug Cole, M.D. (Flagship Ventures)
- Simon Davidson (In-Q-Tel Ventures)
- Dennis Sandstedt (bioMérieux)
All Protein Blood Tests are Based on the Currently Measurable 250 Proteins

<table>
<thead>
<tr>
<th>Serum Concentration</th>
<th>Total Number of Serum Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^{-3}$ M to $1 \times 10^{-12}$ M</td>
<td>250$^1$</td>
</tr>
</tbody>
</table>

How many proteins Are below $1 \times 10^{-12}$ M?

Potential is Large:
Human Proteome = 25,000 genes
Secreted Proteins >2,500

130 FDA Approved Tests$^1$

$^1$Anderson and Anderson, Molecular & Cellular Proteomics 2002: 1.11 845-867
$^2$Klee et al., Nucleic Acids Research 2004: 32, 1414-1421
Single Molecule Arrays (Simoa) Provide a Digital Advantage

**Traditional (Analog)**
- Reaction volume = 100 $\times 10^{-6}$ L
- Diffusion = dilution = low sensitivity
- Millions of molecules needed to reach detection limit

**SimOA (Digital)**
- Reaction volume = 50 $\times 10^{-15}$ L (2 billion times smaller)
- Diffusion defeated = single molecule resolution = ultimate sensitivity
- One molecule needed to reach detection limit
Counting Single Protein Molecules in Blood using Simoa

Rissin et al., Nat. Biotechnol. 2010, 28, 595-599
Kan et al., Lab Chip 2012, 12, 977-985

Quanterix
**Simoa Enables Single Molecule Diagnostics**

**LETTERS**

Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subferomtomolar concentrations

David M. Rissin, Chocheh W. Kim, Todd G. Campbell, Stuart C. Hessel, David R. Fournier, Linan Song, Tomasz Pichl, Purvish Patel, Lei Chang, Andrew J. Rimmah, Evan P. Ferrell, Jeffrey D. Randall, Gail K. Provencher, David R. Walt & David C. Dudley

The ability to detect single protein molecules\(^1\)\(^2\) in blood could accelerate the discovery and use of more sensitive diagnostic biomarkers. To detect low-abundance proteins in blood, we captured them on microscopic beads coated with specific antibodies (one target protein molecule per bead) and then labeled the immunocomplexes with an enzymatic reporter capable of generating a fluorescent product. After loading the beads in a 96-well microwell chamber designed to hold only a single bead, we used fluorescence imaging to detect single protein molecules. Our single-molecule enzyme-linked immunosorbent assay (digital ELISA) approach detected as few as \(10^{-20}\) exosome-labeled complexes in 100 ul of sample \((10^{-21} \text{ M})\) and routinely allowed detection of clinically relevant proteins in serum at concentrations \(10^{-12} \text{ M}\) in much lower concentrations \((10^{-14} \text{ M})\). ELISA detected prostate-specific antigens (PSA) in sera from patients who have undergone radical prostatectomy at concentrations as low as \(34 \text{ fM}\) (0.4 fM).

The clinical use of protein biomarkers is in question in the context of healthy and diseased states, and to monitor disease progression, requires the measurement of low concentrations of proteins in complex samples. Current immunoassays typically measure proteins at concentrations above \(10^{-13} \text{ M}\). The serum concentrations of the majority of proteins are expressed in pmol/L or ng/mL, and the only way to detect protein below this level is to use ultrasensitive immunoassays. To monitor disease progression, ultrasensitive immunoassays are required.

According to the National Cancer Institute, more than 250,000 people are diagnosed with prostate cancer each year in the United States. The most common treatment for prostate cancer is to remove the gland, either through surgery or radiation, and the need for sensitive and accurate tests for detecting prostate cancer is clear.

Using this technology, Simoa demonstrated the detection of 10 ul of PSA in serum\(^3\). This technology has been shown to be successful in detecting proteins at a low concentration in a variety of biological samples, including serum, plasma, urine, and tissue samples. The ability to detect single protein molecules in blood could revolutionize the field of diagnostic testing, allowing for earlier detection and more accurate diagnosis of diseases.

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Simoa Uses Digital Counting and Intensity to Determine Average Number of Enzymes per Bead (“AEB”)

AEB = 0.1  AEB = 0.6  AEB = 3.0

Rissin et al., Anal. Chem. 2011, 83, 2279-2285
Multiplexed Single Molecule Immunoassays

Mixture of subpopulations of encoded capture beads

Capture and enzyme-labeling of multiple, single protein molecules on specific beads

Beads loaded with substrate into arrays of femtoliter wells and sealed with oil

Arrays imaged fluorescently at multiple wavelengths

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06/25/2013
## Digital ELISA Detects Proteins in the fg/mL Range

<table>
<thead>
<tr>
<th>Protein</th>
<th>SiMoA LOD (pg/mL)</th>
<th>Gold Standard Assay LOD (pg/mL)</th>
<th>Vendor</th>
<th>Sensitivity Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.001</td>
<td>0.057</td>
<td>R&amp;D Systems</td>
<td>57</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.003</td>
<td>0.106</td>
<td>R&amp;D Systems</td>
<td>35</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>0.003</td>
<td>0.26</td>
<td>R&amp;D Systems</td>
<td>87</td>
</tr>
<tr>
<td>p24</td>
<td>0.005</td>
<td>15</td>
<td>OCD</td>
<td>3000</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.005</td>
<td>0.039</td>
<td>R&amp;D Systems</td>
<td>8</td>
</tr>
<tr>
<td>PSA</td>
<td>0.006</td>
<td>10</td>
<td>Roche/Siemens</td>
<td>1667</td>
</tr>
<tr>
<td>Aβ42</td>
<td>0.02</td>
<td>50</td>
<td>Innotest</td>
<td>2500</td>
</tr>
<tr>
<td>tau</td>
<td>0.02</td>
<td>60</td>
<td>Innotest</td>
<td>3000</td>
</tr>
<tr>
<td>IL-1α</td>
<td>0.024</td>
<td>1</td>
<td>R&amp;D Systems</td>
<td>42</td>
</tr>
<tr>
<td>GLP-1</td>
<td>0.033</td>
<td>11</td>
<td>Luminex</td>
<td>333</td>
</tr>
<tr>
<td>p-tau (181 + 231)</td>
<td>0.04</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.049</td>
<td>0.115</td>
<td>R&amp;D Systems</td>
<td>2</td>
</tr>
<tr>
<td>troponin</td>
<td>0.05</td>
<td>3</td>
<td>Abbott Architect (HS)</td>
<td>60</td>
</tr>
<tr>
<td>p-tau-231</td>
<td>0.05</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>p-tau-181</td>
<td>0.1</td>
<td>60</td>
<td>Innotest</td>
<td>600</td>
</tr>
</tbody>
</table>
Simoa Addresses Many Opportunities That Exist for High Sensitivity Clinical and Companion Diagnostics

- **Neurology**: Enables blood tests for neurological disease
- **Inflammation**: First to quantify effect of anti-TNF-α drugs on target protein
- **Oncology**: Shorten detection of recurrence (years to months)
- **Cardiac**: World's most sensitive troponin test
- **Infectious Disease**: First IA as sensitive to virus as PCR
- **Metabolism**: First IA as sensitive to virus as PCR
- **Signal Transduction**: HomeBrew
Application Demonstration: Infectious Disease

HIV (p24 capsid protein)

- Achieved 2,000-fold greater sensitivity than benchmark commercial immunoassays (LoD = 5 fg/mL), which is equivalent to about 50 copies of virus per mL. First immunoassay that has shown equivalent sensitivity as nucleic acid testing (NAT).

- Demonstrated full analytical performance (blinded positive and negative sera, blinded seroconversion panels, spiked HIV virus etc.)

- Demonstrated SiMoA is as sensitive as PCR for detecting HIV virus in early infection (seroconversion panels)

- Clinical value: HIV screening for acute infection (prior to seroconversion) and monitoring therapy. Blood screening for infection.

- Commercial leverage: Simplicity, cost and throughput vs. NAT.
Detection of DNA using Simoa

- Detected *S. aureus* spiked into whole blood and Charles River water
- Same sensitivity as PCR (~70 aM)
- Greatly reduced risk of cross-contamination of samples vs. PCR because direct detection of DNA
Simoa HD-1 Analyzer

- Unprecedented ultrasensitivity
- Full automation
- Precision
- Wide dynamic range
- Easy & cost-effective implementation
- Multiplexing capability with small sample volumes
- Homebrew capabilities
- >500 samples per shift
- Available October 2013 for RUO
- IVD clinical studies begin 2014

Sensitivity Means Knowing
Simoa is a high definition diagnostics system that gives you the power to see more clearly, know with certainty, and reach new heights in lab productivity.

Quanterix
Simoa Disc

- Low-cost consumable
- 24 arrays/disc (4 discs = 96 well plate)
- 216,000 femtoliter wells per array
- Manufactured using state-of-the-art Blue-ray™ technology by Sony DADC
- High volume production easily scaleable
Quanterix Instrument Workflow

Step 1. Load Consumables
- Kits (singleplex or multiplex)
- Cuvettes and disposable tips
- Simoa discs

Step 2. Menu Setup
- Select assay
- Calibrators and controls

Step 3. Load Samples
- 96 well plates or tubes

Step 4. Run Samples
- Simoa HD-1 Analyzer performs all mixing, washing, incubation, and sample read-out, with first result in 30 minutes and subsequent samples every 45 seconds thereafter.

- Ultra-sensitive with fully automated simplicity
- Multiplexed analysis and small sample volumes
- Open platform (Homebrew for RUO)
- High volume, low cost production of 24-array discs
Simoa HD-1 Roadmap

2011
Instrument Development

2011-13
Menu Dev.

2013
RUO Launch

2015
Increased plex
DNA, RNA

2016
IVD

SiMoA

Research Use Only

Companion Dx
In vitro diagnostics

Non-regulated clinical studies
Biomarker validation
Pharma, Biotech, Core Labs, CROs
Quanterix Value Map in Biomedical Industry

**Quanterix RUO**

- **Academic Research**
  - Novel Markers

- **Pharma & BioPharma - Clinical**

- **CRO**

**Differentiated Value**

- Sensitivity – Efficiency – Precision – Multiplexing - Value

**Quanterix Revenue Streams**

- Simoa HD-1 Analyzer
- Assay Kits
- Homebrew
- Service
- Boutique Testing & Custom Assay Dev.

**Exclusive Partner**

- Clinical IVD

**Quanterix or Co-Exclusive**

- Blood Screening
- LDTs
- cDx
- POC
- Food Safety, Biothreat, etc.
Thank You