Luminex Platforms and Assays for Effective Detection of Biothreats

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Multiplex Protein Signatures Detection for Biosurveillance

- The surfaces of bacterial cells and viruses are covered in a number of different proteins.
- Each protein in a complex molecule which multiple separate epitopes (antigenic determinants).
- Each epitope can be individually recognized by an antibody molecule.
- **Signature**: a pattern of epitope expression that differentiates one protein or organism from another.

Use multiplex immunoassays to determine signature and ID biothreat.
Benefits of Protein Detection

- **Speed**: Immunoassays are fast and efficient
- **Sensitivity**: Immunoassays are tunable with regards to LOD
  - Longer incubation correlates with lower LOD
- **Specificity**: Multi-signature analysis with epitope mapping
  - Decision tree allows for near neighbor discrimination
- **Cost**: Fairly inexpensive, usually no license burden
- **Automation**: Immunoassays easily automated and extremely amenable to lyophylization
- **Phenotype versus Genotype**: Detection of phenotype covers all the aspects of toxicity
  - Viability, gene expression, and direct detect of toxins
  - Harder to engineer to escape detection – changes that change protein epitopes may render organism non virulent
- **Operational Environment**: Sample prep is significantly less complicated
  - Dilute, concentrate or buffer sample matrix – less sensitive to dirty samples
- **Common platform**: Same platform for environmental and clinical samples
  - Detect host response biomarkers
Luminex internally color-codes microspheres with precise concentrations of two fluorescent dyes.

Red dye
Infrared dye

Bead set has a unique ratio of red and infrared dye.

‘spectral address’
Bead set is identifiable based on red/infrared dye content.

100 distinctly colored bead sets

The bead is impregnated with the dye mixture.
Luminex Microsphere Array

Bead sets can be coated with reagents specific to a particular bioassay, such as antigens, antibodies, oligonucleotides, enzyme substrates or receptors.

MagPlex®
- Superparamagnetic microspheres
- 6.4 microns
- surface carboxyl groups
MAGPIX
Robust FDA-cleared Multiplex xMAP Analyzer

- **Efficiency**
  - Simultaneously measure up to 50 targets in a single reaction

- **Affordability**
  - Affordable multiplexing solution, costs up to 4x less than comparable ELISA assays

- **Flexibility**
  - Analyze both proteins and nucleic acids

- **Smaller footprint**
  - Requires less linear bench space

- **Improved reliability**
  - LED-based illumination and CCD image capture

- **Sensitivity**
  - Approximately $10^6$ copies of DNA or single digit picogram levels of protein

- **Ease of use**
  - Out-of-box user installation
MAGPIX Layout

- Internal Drive Fluid and Waste Bottles
- Optics Module (OM)
  - CCD Camera
  - LED Illumination
  - Imaging Chamber
  - Magnet
  - Probe (XZ axis)
  - Plate Holder (Y Axis)
- Sample Delivery Module (SDM)
Multiplex Immunoassay

Separate Immunoassay on each bead set
### Generation of Classification and Reporter Images

**LED Illumination**
- Capture image with CCD camera and appropriate filter

**Image Analysis**
- Two classification images will identify which bead set each individual bead is from – identifies assay
- Reporter image quantifies assay – is your target analyte present and at what level?

<table>
<thead>
<tr>
<th>Bead Classification</th>
<th>Reporter Image</th>
<th>Assay Reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL1 Filter</td>
<td><img src="image1.png" alt="Classification Image 1" /></td>
<td><img src="image2.png" alt="Assay Image 1" /></td>
</tr>
<tr>
<td>CL2 Filter</td>
<td><img src="image3.png" alt="Classification Image 2" /></td>
<td><img src="image4.png" alt="Assay Image 2" /></td>
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<tr>
<td>RP1 Filter</td>
<td><img src="image5.png" alt="Reporter Image" /></td>
<td><img src="image6.png" alt="Assay Graph" /></td>
</tr>
</tbody>
</table>

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Bead Loading in the MAGPIX Optics Module
Flexibility of Bead-based Arrays

Addition of New Targets to Assay Panel

Biothreat Multiplex Assay Panel

New target identified

Assay built on additional bead set

New assay added to panel

• Bacillus anthracis (BA)
• Yersinia pestis (YP)
• Francisella tularensis (FT)
• Botulinum toxin (BoT)
• Staphylococcus enterotoxin B (SEB)

Simultaneous Detection of Proteins and Nucleic Acids

Potential applications:

• **Environmental**: detect nucleic acid of agent and direct detect toxins and agents (multi-orthogonal approach)

• **Clinical**: detect NA of infectious disease agent and determine host response to infection

Benefits:

• Sensitivity inherit to nucleic acid assays
• Detect non nucleic-acid compounds – such as BW toxins
Benefits of Multi-signature Analysis

Detection of multiple epitopes of a protein target

- Protect against genetic drift or shift (natural or intentional)
- Extend the dynamic range of the assay
- Decrease false alarm rate
Luminex MAGPIX optics module is integrated into autonomous environmental detection system
- Greater than TRL6+
- Main barrier to implementation is lack of funding to test systems in the field
- High performance multiplex PCR assay with xMAP endpoint read
  - *Tested extensively by third parties*
- System extremely reliable – eliminate downtime through hot swap spares – more cost effective

Could easily implement protein assays on same system
- Could be TRL6+ by 2016
- No major technology issues
- Integration issues
  - *Need to demonstrate equivalent immunoassay performance on autonomous system that is seen with benchtop testing*
  - *Deployment of stable reagents and ensure performance over entire service period*
Example Sensitivities of Immunoassays for Biosurveillance

<table>
<thead>
<tr>
<th>Threat Agents</th>
<th>Simulants</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. anthracis</td>
<td>B. globigii</td>
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<tr>
<td>~1 x 10^3 cfu/mL</td>
<td>~1 x 10^3 cfu/mL</td>
</tr>
<tr>
<td>Y. pestis</td>
<td>MS2</td>
</tr>
<tr>
<td>~1 x 10^3 cfu/mL</td>
<td>~1 x 10^5 pfu/mL</td>
</tr>
<tr>
<td>F. tularensis</td>
<td>E. herbicola</td>
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<tr>
<td>~1 x 10^3 cfu/mL</td>
<td>~5 x 10^3 cfu/mL</td>
</tr>
<tr>
<td>V. cholera</td>
<td>Ovalbumin</td>
</tr>
<tr>
<td>~3 x 10^3 cfu/mL</td>
<td>~ 10 pg/mL</td>
</tr>
<tr>
<td>Brucella (sp.)</td>
<td></td>
</tr>
<tr>
<td>~3 x 10^3 cfu/mL</td>
<td></td>
</tr>
<tr>
<td>B. mallei (glanders)</td>
<td></td>
</tr>
<tr>
<td>R. pseudomallei</td>
<td></td>
</tr>
<tr>
<td>Orthopox</td>
<td></td>
</tr>
<tr>
<td>~1 x 10^2 pfu/mL</td>
<td></td>
</tr>
<tr>
<td>VEE</td>
<td></td>
</tr>
<tr>
<td>~1 x 10^5 pfu/mL</td>
<td></td>
</tr>
<tr>
<td>WEE</td>
<td></td>
</tr>
<tr>
<td>EEE</td>
<td></td>
</tr>
<tr>
<td>SEB</td>
<td>10 pg/mL</td>
</tr>
<tr>
<td>SEA</td>
<td>10 pg/mL</td>
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<tr>
<td>SEC</td>
<td>10 pg/mL</td>
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<tr>
<td>Ricin</td>
<td>1 pg/mL</td>
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<tr>
<td>Abrin</td>
<td>10 pg/mL</td>
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<tr>
<td>BoNT-A</td>
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</tr>
<tr>
<td>BoNT-B</td>
<td>~30 pg/mL</td>
</tr>
<tr>
<td>BoNT-C</td>
<td>~30 pg/mL</td>
</tr>
<tr>
<td>BoNT-D</td>
<td>~30 pg/mL</td>
</tr>
<tr>
<td>BoNT-E</td>
<td>~30 pg/mL</td>
</tr>
<tr>
<td>BoNT-F</td>
<td>~30 pg/mL</td>
</tr>
<tr>
<td>BoNT-G</td>
<td>~30 pg/mL</td>
</tr>
</tbody>
</table>

Sensitivity:

- Sensitivity not expressed as number of copies but as organisms or picogram per ml
- Sensitivity dependent on prevalence of protein epitope (detection of highly expressed surface protein may allow for lower LOD in terms of organisms detected)
Thank You

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