Multi-Array™ Immunodetection of Biowarfare Agents Using the Meso Scale Discovery ™ Sector PR™

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ECBC Bioanalysis

- Current bioanalysis roadmap supports an integrated approach for detection and identification using multiple technologies

- We have combined state-of-the-art immunoassay analysis (ORIGEN® ECL) and nucleic acid analysis (SmartCycler® Taqman™)

- Our current bioanalysis approach employs a tiered structure
  - Tier 1 : Presumptive ID by immunoassay
  - Tier 2 : Pathogen confirmation by PCR/Cell culture
    Toxin confirmation by MS

Immunoassay Challenges

- Front-end immunoanalysis requires development of sensitive & specific assays for presumptive screening of bioagents
  - Assay sensitivity cannot compromise assay specificity
    - Set assay cutoffs and LODs
    - Validate each assay for the matrix being analyzed
    - Cutoff must be above any sample matrix effects
    - Target sensitivities within the LD_{50} and infective dosage ranges
  - Assay specificity has been an issue for some immunoassays
    - Need affinity-based antibodies directed to specific targets
    - Need to overcome sample matrix issues
    - Need to investigate alternative front-end sample clean up methodologies

Immunoassay Challenges, cont.

- Identify technologies that feature simultaneous analyses of samples for multiple targets
  - Demonstrate mobility and simplicity for field use, urban monitoring, and mobile labs
  - Identify reliable, simple, rapid, and cost effective immunoassays
- Identify technologies that yield higher throughput analysis

Electrochemiluminescence

- ECL technology is elegant and yields exquisite sensitivity
- ECL measurement is simple and rapid
- Ruthenium tris (bipyridyl) $Ru(bpy)_3^{2+}$ is a small molecule (< 1000 Da)
- Coupling chemistry is easy and straightforward
- $Ru(bpy)_3^{2+}$ is an extremely stable compound

ECBC ECL FASTube History

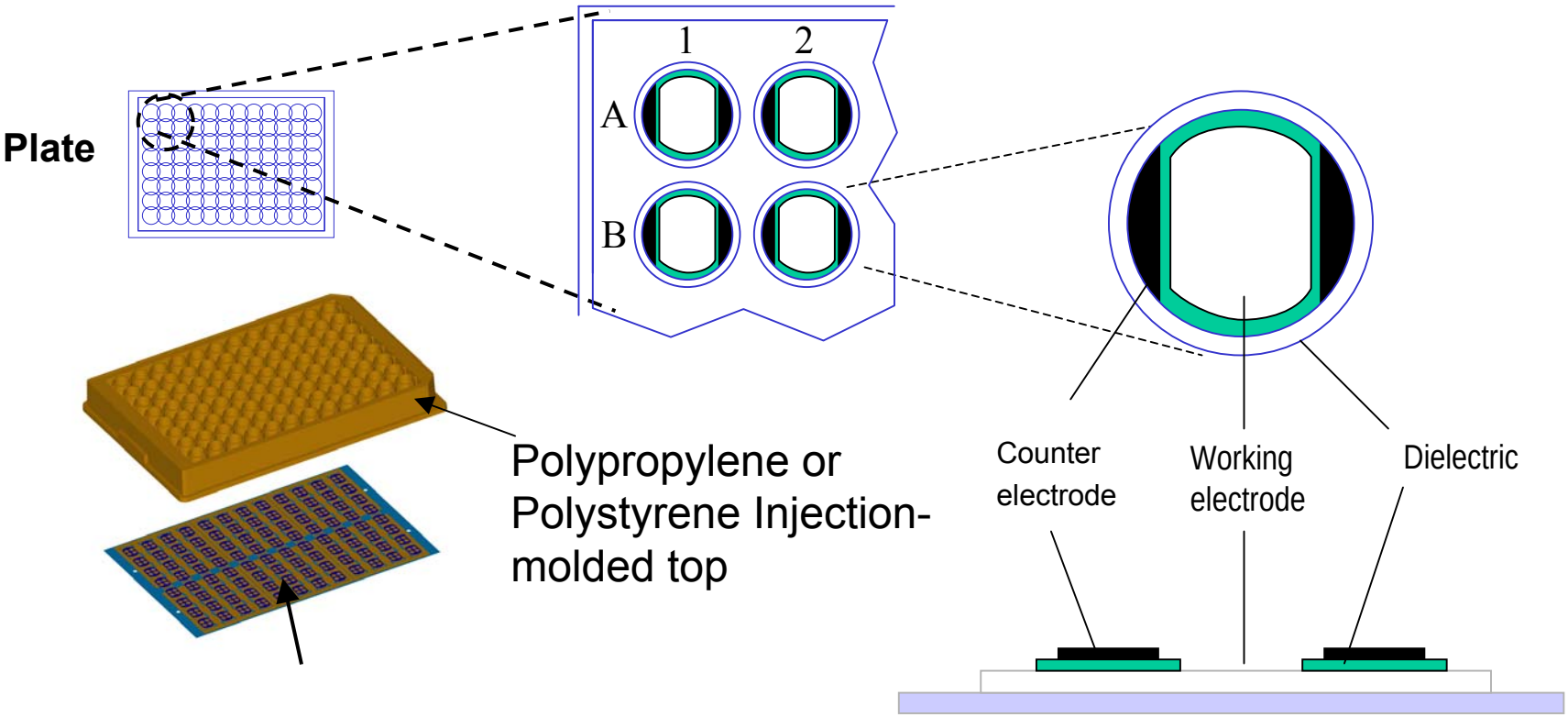
FY95-96	FY97-98	FY99	FY00	FY01-03
• R&D Evaluation COTS • Optimize wet chemistry • Publish results • Develop one-step ECL assay • Dry down reagents • JFT assessment	FASTube validation	• U.S. Patent Application filed for method and device • FASTube transition to TEU	FASTube technology transition to USAMRIID	• 2001 FASTube technology transitions to JPEO-CBD • 2002 full-scale FASTube production by IGEN • 2003 FASTube assays support Homeland Defense, TAML, TEU, OGAs, Iraqi Freedom • 2003 FASTube assays offered in JPEO-CBD Reagent Catalog

Sector PR™ Pattern Array ECL Technology

- The Sector PR™ is a compact ECL reader designed by Meso Scale Discovery™ (MSD)
- The Sector PR™ is shoebox-sized and reads ECL signals from a 96 well plate in less than two minutes

Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.

Multi-Array™ Technology: Printed Pattern Electrodes



Screen Printed High Density Electrode Arrays

- Arrays are made from several patterned layers including layers of conducting carbon inks for the electrodes

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Objectives

- Evaluate Sector PR™ ECL assays:
  - Assay endpoint sensitivities and dynamic range
  - Intra- and Inter-assay variation
  - Assay specificity
  - Analysis of environmental samples (ENV)
  - Artificially spiked ENV samples

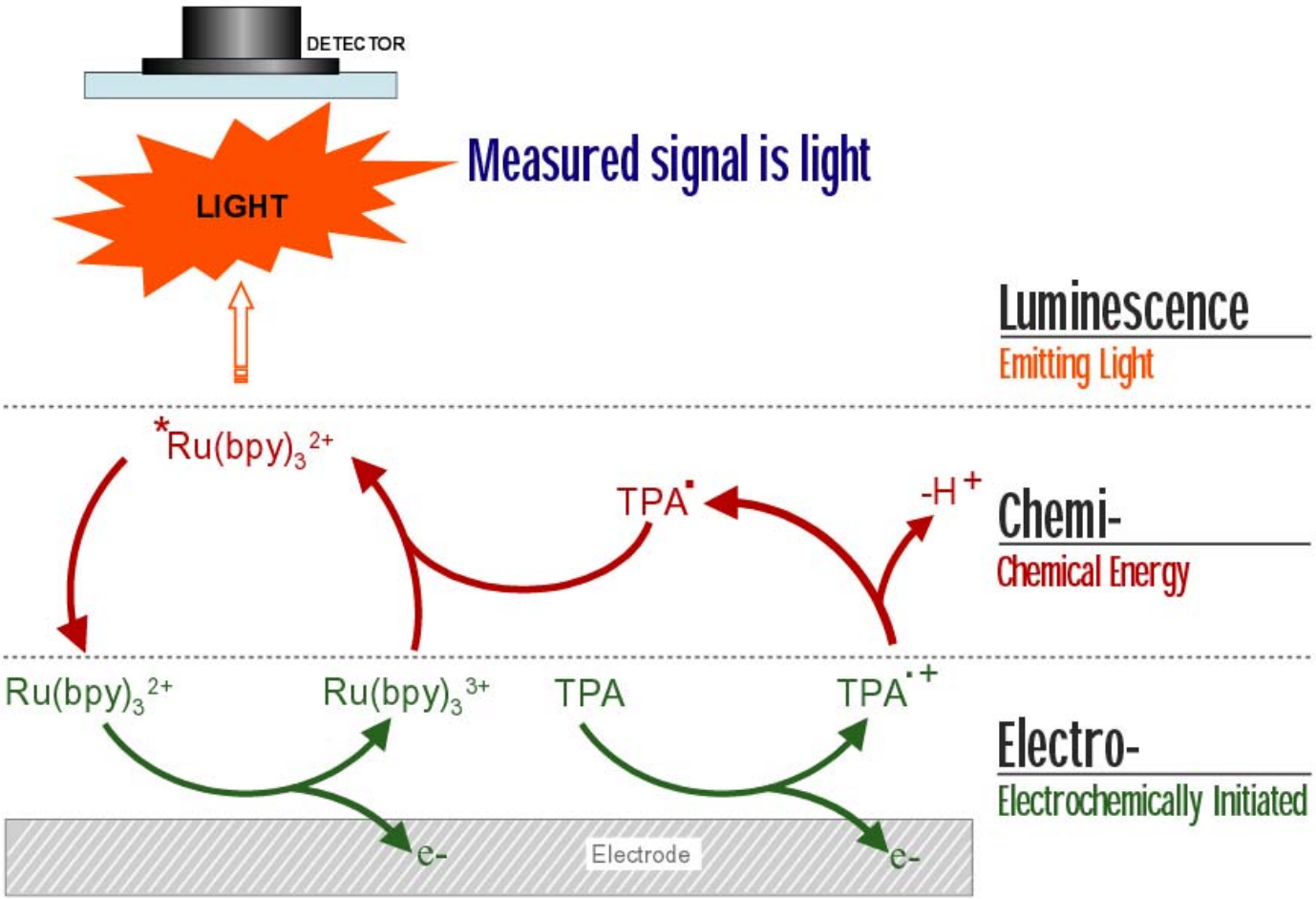
- Demonstrate proof-of-principle Multi-Array™ PR immunoassays
  - Staphylococcal enterotoxin B toxin
  - *Bacillus anthracis* spores
  - *Yersinia pestis,* F1
  - Venezuelan Equine Encephalitis

- Compare Sector PR™ results to the gold standard assays conducted on the ORIGEN® 1.5 ECL sensor

Reaction chemistries

- Immobilize biotin conjugated capture antibody onto MSD streptavidin-coated 96 well carbon electrode plates
- Add ruthenium conjugated detector antibody and antigen to form an immunocomplex on the surface of the carbon electrode
- Apply voltage to the electrodes in the presence of the co-reactant tripropylamine (TPA)
- Eight silicon diodes sense the release of photons generated from the applied potential to the electrode, which is converted to the ECL signal

ECL Detection



MSD
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Materials and Methods

- Antibodies:
  - Government furnished antibodies
  - Antibody conjugates
    - biotinylated capture antibodies
    - detector antibody labeled with ruthenium tris bipyridyl

- Format:
  - Mab anti-SEB capture / Pab rabbit anti-SEB detector
  - Mab anti-*B. anthracis* capture / Pab rabbit anti-*B. anthracis* detector
  - Mab anti-VEE capture / Mab anti-VEE detector
  - Mab anti-*Y. pestis* capture / Pab rabbit anti-*Y. pestis* detector

Materials and Methods, cont.

Antigens:

- SEB toxin, Sigma, (0 -100 ng/ml)
- Gamma-inactivated *Bacillus anthracis*, Ames strain, AFIP, (0 - 1 x 10^6 cfu/ml)
- Gamma-inactivated VEE virus,TC-83 strain, USAMRIID, (0 - 1 x 10^7 pfu/ml)
- Gamma-inactivated *Yersinia pestis*, India strain, DPG, (0 - 1 x 10^6 cfu/ml)

Materials and Methods, cont.

Assay Optimization:

- Capture antibodies matrixed from 30 -120 ng/test
- Detector antibodies were matrixed in the same manner as the capture
- The optimal capture/detector antibody concentration was determined through examination of endpoint sensitivity, CV, and dynamic range within the 15 -minute assay timeframe

Materials and Methods, cont.

Assay protocol:

- Coat MSD Streptavidin-Coated Multi-Array™ PR plate wells with 25 μl of capture antibody
- Incubate at 4°C ON and wash three times in 0.01M PBS prior to analysis
- Add 25 μl of detector antibody
- Add 100 μl of the analyte to well and incubate for 15 min
- Wash plate three times with 0.01M PBS
- Add 150 μl of 1X MSD Read Buffer to each of the wells
- Read plate on the Sector PR

Sector PR™ Washed Assay Data

Background + 3x STD: 927 ECL Units
Lower Limit of Detection: 0.01 ng/ml
SEB MSD Washed Assay
60 ng biotin / 50 ng ruthenium per test

ng/mL	ECL Average Raw Signal	ECL Specific Signal	% CV	S/N
0	859	-68	2.6	1.0
0.001	831	-96	3.5	1.0
0.005	891	-36	3.4	1.0
0.010	1043	116	2.5	1.2
0.10	3005	2078	2.3	3.5
1.0	23356	22429	1.2	27.2
10.0	222535	221608	3.7	259.1
100.0	554304	553377	15.8	645.3

Background + 3x STD: 704 ECL Units
Lower Limit of Detection: 5.0E+04 cfu/ml
B. anthracis MSD Washed Assay
90 ng biotin / 100 ng ruthenium per test

cfu/mL	ECL Average Raw Signal	ECL Specific Signal	% CV	S/N
0	593	-111	6.2	1.0
1.0E+01	563	-141	2.8	0.9
1.0E+02	599	-105	9.9	1.0
1.0E+03	586	-118	6.3	1.0
1.0E+04	651	-53	5.1	1.1
5.0E+04	741	37	2.0	1.3
1.0E+05	880	176	2.9	1.5
1.0E+06	2306	1602	4.3	3.9

Background + 3x STD: 802 ECL Units
Lower Limit of Detection: 5.0E+06 pfu/ml
VEE MSD Washed Assay
30 ng biotin / 100 ng ruthenium per test

pfu/mL	ECL Average Raw Signal	ECL Specific Signal	% CV	S/N
0	716	-86	4.0	1.0
1.0E+03	702	-100	4.8	1.0
1.0E+04	730	-72	10.5	1.0
1.0E+05	717	-85	4.4	1.0
5.0E+05	752	-49	5.3	1.1
1.0E+06	714	-88	1.7	1.0
5.0E+06	975	173	11.5	1.4
1.0E+07	1055	253	2.4	1.5

Background + 3x STD: 843 ECL Units
Lower Limit of Detection: 1.0E+03 cfu/ml
Y. pestis MSD Washed Assay
60 ng biotin / 100 ng ruthenium per test

cfu/mL	ECL Average Raw Signal	ECL Specific Signal	% CV	S/N
0	777	-66	2.8	1.0
1.0E+01	792	-51	3.1	1.0
1.0E+02	809	-34	4.2	1.0
1.0E+03	938	95	2.5	1.2
5.0E+03	1570	727	0.4	2.0
1.0E+04	2392	1549	1.5	3.1
1.0E+05	14349	13506	1.5	18.5
1.0E+06	58640	57797	3.5	75.4

Comparison of Platform Tradeoffs

		ORIGEN 1.5®	Sector PR™
Sensitivity			
	B. anthracis	5 x 10^3 cfu/ml	5 x 10^4 cfu/ml
	Y. pestis	1 x 10^2 cfu/ml	1 x 10^3 cfu/ml
	VEE	5 x 10^5 pfu/ml	1 x 10^6 pfu/ml
	SEB	5 pg/ml	10 pg/ml
Tradeoffs			
	Incubation Time	15 min	15 min
	Read Time	75 min	2 min
	# of Determinations	50	96
	Footprint	12” x 12” x 16”	8.6” x 8.8” x 16”
	Weight	47 lbs.	18 lbs.
	In-house cost per assay	$5 – $7	< $2

Multi-Array™ PR Crossreactivity Results

Anthrax challenge

	SEB			*B. anthracis*			VEE			*Y. pestis*		
Bkgd	618	627	599	**Flyer**	**640**	**689**	625	676	806	935	917	997
5e04 cfu/ml	585	653	637	**873**	**823**	**778**	681	624	697	882	853	869
1e05 cfu/ml	632	587	557	**921**	**964**	**924**	667	606	674	875	888	862
1e06 cfu/ml	643	593	711	**2436**	**2195**	**2256**	658	622	642	892	894	896
Bkgd	**612**	**625**	**623**	598	612	682	592	601	619	767	738	777
0.010 ng/ml	**775**	**796**	**789**	651	699	691	700	670	687	893	847	913
1 ng/ml	**22598**	**19526**	**20056**	692	714	719	627	726	732	923	952	917
100 ng/ml	**677958**	**658647**	**634486**	699	783	804	767	732	754	890	933	958

SEB challenge

Crossreactivity Results, cont.

VEE challenge

	SEB			*B. anthracis*			VEE			*Y. pestis*		
Bkgd	531	594	531	681	614	625	**608**	**600**	**620**	763	763	779
1e06 pfu/ml	585	593	598	673	675	671	**669**	**667**	**685**	770	785	770
5e06 pfu/ml	644	636	610	678	675	611	**872**	**880**	**880**	801	814	822
1e07 pfu/ml	607	572	523	679	642	631	**984**	**1000**	**979**	760	730	836
Bkgd	545	586	539	617	637	676	621	673	617	**738**	**766**	**792**
1e03 cfu/ml	557	605	550	664	656	646	605	618	651	**1046**	**1089**	**1105**
1e04 cfu/ml	567	608	597	697	669	698	679	676	670	**3502**	**3552**	**3640**
1e06 cfu/ml	508	573	575	645	648	629	658	659	610	**111711**	**109926**	**117330**

Y. pestis challenge

Environmental Sample Analysis

Multi-Array™ PR Plate Set Up

ENV sample	SEB			*B. anthracis*			VEE			*Y. pestis*		
Neg. Cntrl.	386	396	389	393	462	428	376	385	413	548	524	465
ENV 1	452	398	402	498	445	501	439	457	420	609	640	597
ENV 2	396	383	347	436	425	437	329	392	340	571	560	499
ENV 3	482	450	399	488	503	487	400	433	416	628	644	644
ENV 4	491	497	418	469	516	475	437	457	473	654	629	620
ENV 1 Spike	**25316**	**25705**	**24561**	**1661**	**1673**	**1709**	**997**	**1059**	**1019**	**3065**	**2940**	**2897**
ENV 3 Spike	**23819**	**23209**	**22361**	**1579**	**1569**	**1482**	**827**	**901**	**826**	**2779**	**2728**	**2697**
Pos. Cntrl.	**23224**	**22839**	**22499**	**1642**	**1553**	**1466**	**1248**	**1297**	**1254**	**2490**	**2381**	**2484**

Environmental Sample Analysis Results

SEB

Day 1

Sample	ECL Raw Signal	S/N	Result
buffer negative	371	1.0	-
ENV-1	418	1.1	-
ENV-2	379	1.0	-
ENV-3	449	1.2	-
ENV-4	455	1.2	-
ENV-1 spiked	30181	81.3	+
ENV-3 spiked	28281	76.2	+
positive control	27099	73.0	+

Day 2

Sample	ECL Raw Signal	S/N	Result
buffer negative	390	1.0	-
ENV-1	417	1.1	-
ENV-2	375	1.0	-
ENV-3	444	1.1	-
ENV-4	469	1.2	-
ENV-1 spiked	25194	64.5	+
ENV-3 spiked	23130	59.3	+
positive control	22854	58.5	+

Day 3

Sample	ECL Raw Signal	S/N	Result
buffer negative	402	1.0	-
ENV-1	483	1.2	-
ENV-2	403	1.0	-
ENV-3	494	1.2	-
ENV-4	499	1.2	-
ENV-1 spiked	16942	42.1	+
ENV-3 spiked	16044	39.9	+
positive control	19064	47.4	+

B. anthracis

Day 1

Sample	ECL Raw Signal	S/N	Result
buffer negative	446	1.0	-
ENV-1	471	1.1	-
ENV-2	449	1.0	-
ENV-3	505	1.1	-
ENV-4	494	1.1	-
ENV-1 spiked	1623	3.6	+
ENV-3 spiked	1701	3.8	+
positive control	1758	3.9	+

Day 2

Sample	ECL Raw Signal	S/N	Result
buffer negative	428	1.0	-
ENV-1	481	1.1	-
ENV-2	433	1.0	-
ENV-3	493	1.2	-
ENV-4	487	1.1	-
ENV-1 spiked	1681	3.9	+
ENV-3 spiked	1543	3.6	+
positive control	1554	3.6	+

Day 3

Sample	ECL Raw Signal	S/N	Result
buffer negative	476	1.0	-
ENV-1	525	1.1	-
ENV-2	435	0.9	-
ENV-3	502	1.1	-
ENV-4	509	1.1	-
ENV-1 spiked	1621	3.4	+
ENV-3 spiked	1549	3.3	+
positive control	1529	3.2	+

Environmental Sample Analysis Results

VEE

Day 1

Sample	ECL Raw Signal	S/N	Result
buffer negative	391	1.0	-
ENV-1	420	1.1	-
ENV-2	403	1.0	-
ENV-3	437	1.1	-
ENV-4	420	1.1	-
ENV-1 spiked	929	2.4	+
ENV-3 spiked	836	2.1	+
positive control	1165	3.0	+

Day 2

Sample	ECL Raw Signal	S/N	Result
buffer negative	391	1.0	-
ENV-1	439	1.1	-
ENV-2	354	0.9	-
ENV-3	416	1.1	-
ENV-4	456	1.2	-
ENV-1 spiked	1025	2.6	+
ENV-3 spiked	851	2.2	+
positive control	1266	3.2	+

Day 3

Sample	ECL Raw Signal	S/N	Result
buffer negative	427	1.0	-
ENV-1	489	1.1	-
ENV-2	348	0.8	-
ENV-3	465	1.1	-
ENV-4	478	1.1	-
ENV-1 spiked	984	2.3	+
ENV-3 spiked	837	2.0	+
positive control	1457	3.4	+

Y. pestis

Day 1

Sample	ECL Raw Signal	S/N	Result
buffer negative	562	1.0	-
ENV-1	611	1.1	-
ENV-2	554	1.0	-
ENV-3	627	1.1	-
ENV-4	641	1.1	-
ENV-1 spiked	3040	5.4	+
ENV-3 spiked	2920	5.2	+
positive control	2693	4.8	+

Day 2

Sample	ECL Raw Signal	S/N	Result
buffer negative	512	1.0	-
ENV-1	615	1.2	-
ENV-2	543	1.1	-
ENV-3	639	1.2	-
ENV-4	634	1.2	-
ENV-1 spiked	2967	5.8	+
ENV-3 spiked	2735	5.3	+
positive control	2452	4.8	+

Day 3

Sample	ECL Raw Signal	S/N	Result
buffer negative	583	1.0	-
ENV-1	684	1.2	-
ENV-2	549	0.9	-
ENV-3	814	1.4	-
ENV-4	717	1.2	-
ENV-1 spiked	3859	6.6	+
ENV-3 spiked	3741	6.4	+
positive control	2935	5.0	+

Wrap Up

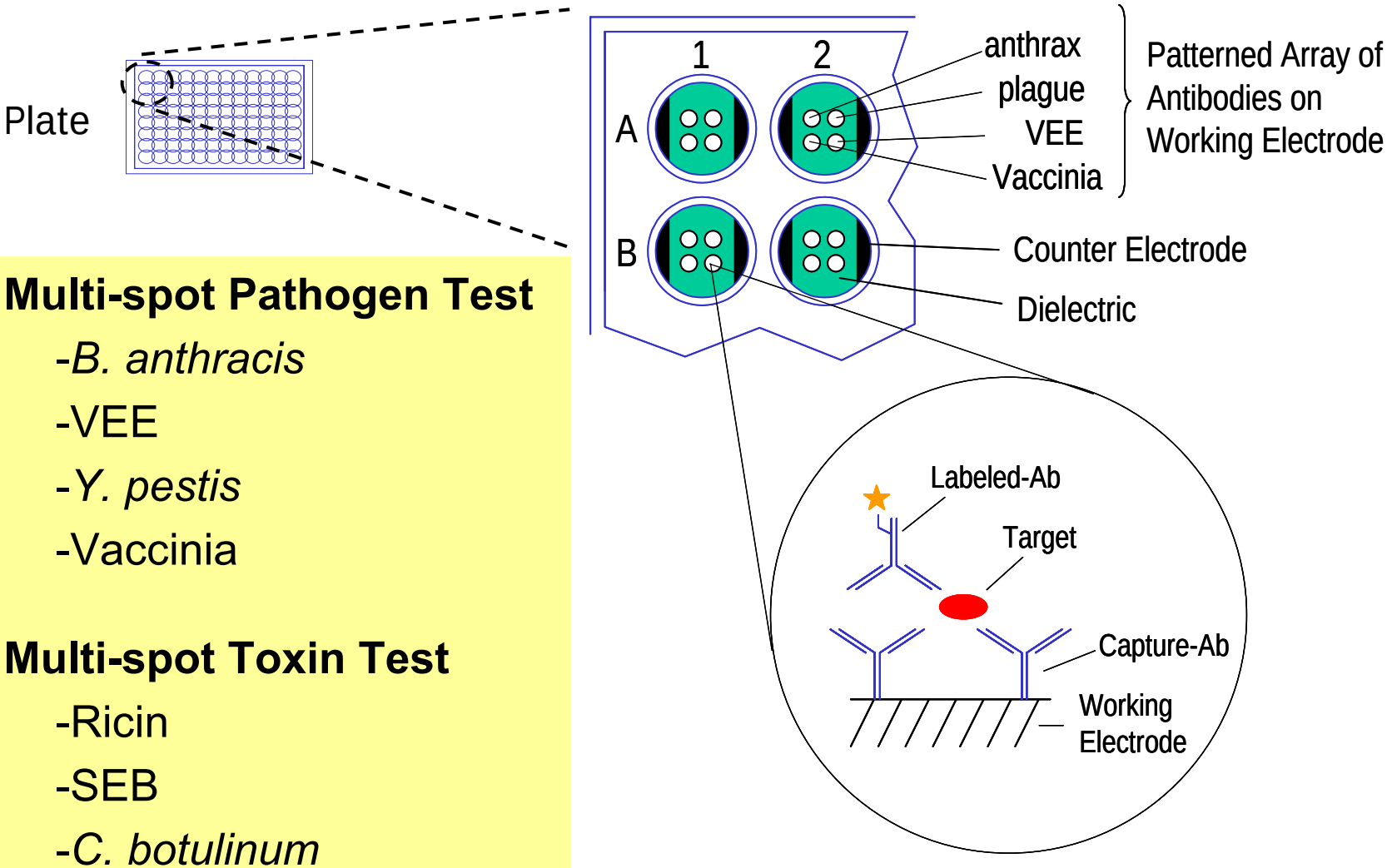
- We have developed four 15-minute washed assays on the Sector PR, a novel bioassay platform from Meso Scale Discovery
- Assays demonstrated excellent sensitivity and specificity
- Overall sensor performance was comparable to the ORIGEN® 1.5
- Exquisite CV's were achieved - below 5% in most cases
- Assay protocols have been developed for both washed and nonwashed formats. The nonwashed format is under development

Wrap Up, cont.

- ENV samples were challenged with four antigens in Multi-Array fashion
- Cross-reactivity studies performed on the Multi-Array plates showed no evidence of nonspecific interaction
- The Sector PR assays are very sensitive, robust, and cost effective
- The Sector PR is shoe-box sized, is light and portable, and has no fluidics

Future Objectives

- **Multi-spot Pathogen Test**
  - -*B. anthracis*
  - -VEE
  - -*Y. pestis*
  - -Vaccinia
- **Multi-spot Toxin Test**
  - -Ricin
  - -SEB
  - -*C. botulinum*



Future Objectives, cont.

- Investigate feasibility of dried-down reagents in MSD 96-well plates using StabilCoat® buffer and lyophilization buffer
- Proceed with assay development of additional biowarfare agents
- Refine wash and nonwashed assay formats
- Continue to evaluate MSD next generation bioanalysis platforms
- Evaluate sensor potential for portability, ease of use, sensitivity, and speed

Technology Watch

Instrument

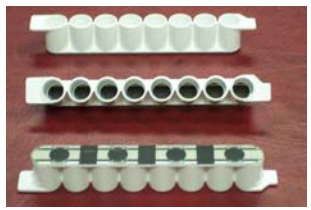
Ruggedized Sector PR

Strip Reader

Cartridge Reader

Consumables

96-Well Plate, 4-Spot Plate, Multiple Strips of Wells. Dried reagents in wells



Single Strip of Wells. Dried reagents in wells

Cartridge contains all consumables

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