

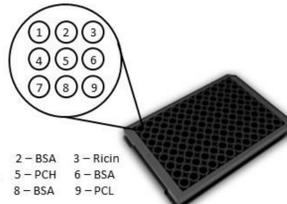
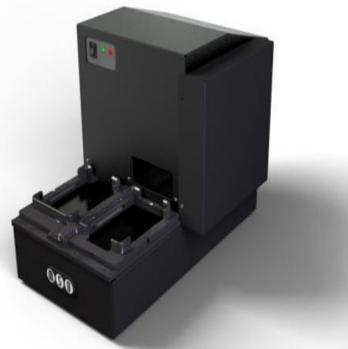
Determining the Performance Specifications of Multiplex Biothreat Assays for the National Guard ALS Vehicle

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The biological toxin identification component of the existing Analytical Laboratory System 1 (ALS 1), the BioVeris M1M Electrochemiluminescence Bioanalyzer (M1M), became obsolete at the end of calendar year 2013 and is no longer supported by the vendor; thus the CBRNE A&RS has an urgent and critical need to replace the M1M in each ALS with an appropriate alternative biological analyzer. The Critical Reagents Program (CRP) has designated the BioSciences Division within ECBC to function as an independent testing lab for the characterization of the multiplex assay plate and reagents for the Meso Scale Defense (MSD) PR2 Model 1800 platform.

Phase III multiplex characterization testing was performed with aqueous suspensions of one active toxin (Bot A Toxin In-Complex) and two inactivated toxins (Ricin A Chain and SEBv recombinant toxoid) prepared in matrices selected by ALS. Each assay was assessed based on performance of Assay Linearity, Limit of Detection (LOD), Sensitivity (Inclusivity), and Specificity (Exclusivity and Cross-Reactivity).

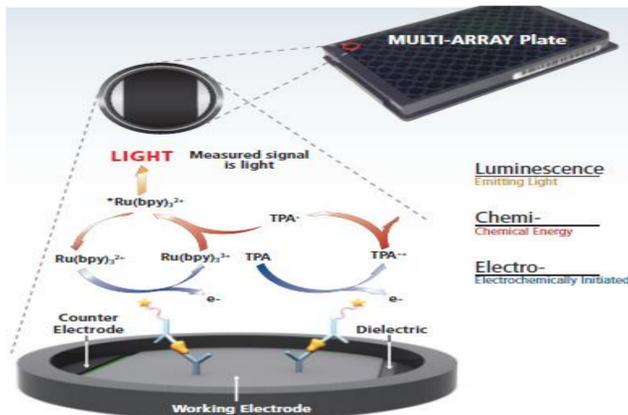


Electrochemiluminescence (ECL)

ECL detection utilizes labels that emit light when electrochemically stimulated. Background signals are minimal because the stimulation mechanism (electricity) is decoupled from the signal (light).

Labels are stable, non-radioactive, and offer a choice of convenient coupling chemistries. They emit light at ~620nm, eliminating problems with color quenching. Few compounds interfere with the electrochemiluminescent labels, so large, diverse libraries can be used with confidence.

Multiple excitation cycles of each label amplify the signal to enhance light levels and improve sensitivity.

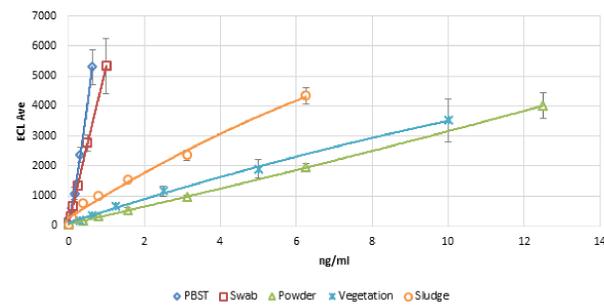


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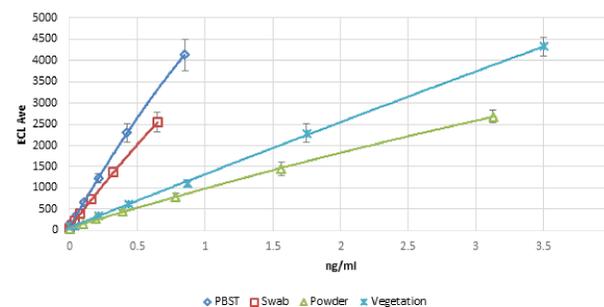
Assay Linearity

The linearity of each multiplex assay for each antigen was assessed in order to determine the dose response curve of the assay, the amount of variability at various antigen concentrations, and the ability of the assay to quantify results. This LOD served as the starting point for the complete evaluation of the LOD.

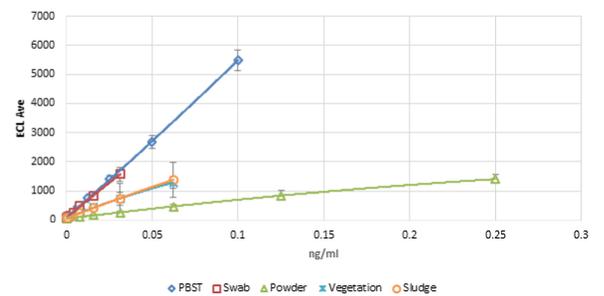
Ricin Assay Linearity



Bot Assay Linearity



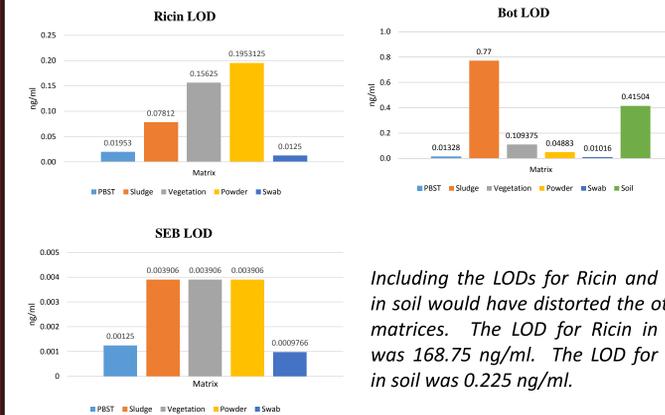
SEB Assay Linearity



Data for soil for all three assays (as well as sludge for Bot) was excluded because it would have distorted other matrices.

Limit of Detection

Using the estimated or conventional LOD established in the assay linearity study, an actual LOD was established by running test samples spiked with target antigen in each matrix at assay-specific concentrations.



Inclusivity

Inclusivity testing included LOD range-finding tests with near neighbors and replicate testing at the LOD for each analyte. Inclusivity testing was performed at 1X LOD and 10X LOD.

Ricin			Bot		
Organism	Concentration Tested (ng/ml)	Avg S/N	Organism	Concentration Tested (ng/ml)	Avg S/N
Ricin A Chain	0.025	3.0	Bot A In-Complex	0.05	4.4
Ricin A Chain	0.05	4.7	Bot A In-Complex	0.5	36.7
Ricin A Chain	0.1	9.6	Bot A Toxoid	0.05	2.1
Ricin A Chain	0.25	23.5	Bot A Toxoid	0.5	11.6
Ricin RCA 120	0.025	1.2	Bot A Crude Extract	0.05	4.5
Ricin RCA 120	0.05	1.6	Bot A Crude Extract	0.5	34.0
Ricin RCA 120	0.1	2.2	Bot A Pure	0.05	1.2
Ricin RCA 120	0.25	4.0	Bot A Pure	0.5	1.9
Ricin RCA 60	0.025	2.9	Bot B Pure	0.05	1.7
Ricin RCA 60	0.05	4.7	Bot B Pure	0.5	5.4
Ricin RCA 60	0.1	10.0			
Ricin RCA 60	0.25	18.7			
Ricin Toxoid	0.025	1.5			
Ricin Toxoid	0.05	2.5			
Ricin Toxoid	0.1	4.0			
Ricin Toxoid	0.25	6.7			

Because Ricin RCA 120 and Ricin Toxoid were not detected at 1X LoD in all experiments, Ricin inclusivity was performed again using 0.05 ng/ml and 0.1 ng/ml.

SEB		
Organism	Concentration Tested (ng/ml)	Avg S/N
SEB Toxin	0.00125	2.0
SEB Toxin	0.0125	17.9
S. aureus 14458	1x10 ⁴ cfu/ml	26.6
S. aureus 14458	1x10 ⁴ cfu/ml	277.1
S. aureus 14458	1x10 ⁴ cfu/ml	2774.0
SEBv	0.00125	2.5
SEBv	0.0125	9.3

Exclusivity

Exclusivity determined whether or not an assay demonstrated cross-reactivity with other closely-related or environmentally-relevant organisms. Cross-Reactivity determined whether or not an assay reacted with organisms targeted by the other assays. Exclusivity and Cross-Reactivity testing were performed at 1,000X LOD.

Organism	Concentration Tested (ng/ml)	Ricin	Bot	SEB
		1,000X LoD	1,506X LoD	16,000X LoD
Abrin	20	1.0	1.1	0.9
Alpha Toxin	20	0.9	1.2	1.7
Bot E Pure Neurotoxin	20	1.0	1.2	1.1
Clostridium difficile Toxin A	20	1.0	1.1	1.3
Diphtheria Toxoid	20	0.9	1.1	1.2
Gelolin	20	0.9	1.0	0.9
Ovalbumin	20	1.0	1.2	1.4
Phytolacca americana Lectin	20	1.0	1.2	1.9
Viscum Album Lectin	20	1.0	1.2	1.0
Saporin	20	0.9	1.1	1.1
Shiga Toxin	20	0.8	1.0	0.9
Tetanus Toxin	20	0.9	1.1	1.0
Thermolysin	20	0.9	1.1	1.0
Toxic Shock Syndrome Toxin	20	1.0	1.5	1.6
SEA Toxin	20	1.1	1.1	1.0
SEC1 Toxin	20	0.9	1.1	25.7
Ricin RCA 60	20	1541.3	1.3	1.7
Ricin RCA 120	20	247.5	1.1	0.9
Ricin A Chain	20	2196.4	1.4	1.5
Ricin B Chain	20	1.0	1.0	0.9
Ricin Toxoid	20	502.4	1.2	1.0
SEB Toxin	20	2.0	2.1	10014.3
Staphylococcal aureus 14458	20	0.8	1.0	24.2
SEBv	20	1.4	1.9	9499.4
Bot A Pure	20	0.9	27.6	0.9
Bot B Pure	20	0.9	178.8	0.9
Bot A Crude	20	0.9	347.5	0.9
Bot A Complex	20	0.8	872.3	1.1
Bot 62A	20	0.8	79.4	1.2
Bot Toxoid	20	0.9	295.0	0.9

Overall Results

Phase III multiplex studies generated LODs 13-75 times more sensitive in PBST than the M1M for the three toxin assays. The multiplex assay plates increase sample throughput while providing greater sensitivity.

Acknowledgements

Dr. Michael A. Smith, Ph.D.
Director, CRP
Mr. Bruce Goodwin
Deputy Director, CRP
Ms. Leigh Anne Alexander
Science Manager, CRP

