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R E S E A R C H R E P O R T

Evaluation Report on the QTL Biosystems *Bacillus anthracis* Cartridges and β-unit Reader

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Background

A comprehensive evaluation of the QTL BioSystems beta-test unit with the *Bacillus anthracis* cartridge was performed. The evaluation encompassed system performance, sensitivity, specificity, and robustness testing for white powders. Testing on *B. anthracis* near neighbors and vaccine strains was performed in the biosafety level-2 (BSL2) laboratory as was examination of the effects of powder matrix on non-pathogenic anthrax (*B. anthracis* Pasteur) detection. Final evaluation on pathogenic *B. anthracis* was performed in the BSL3 laboratory. Subsequent vapor hydrogen peroxide (VHP) decon of the unit was performed and repeated analysis of non-pathogenic *B. anthracis* was performed to evaluate the effect of VHP exposure on system performance.

Results and Discussions

I- Specificity

To evaluate assay specificity, high concentrations (>10^5 organisms) of near neighbor or other potential biothreat organism were assayed on the β unit employing the *B. anthracis* specific assay cartridges. Results are shown in Table 1.

Table 1. Analysis of near neighbors and other potential biothreat agents in *B. anthracis* cartridge. A positive result was defined as a response (mV) greater than 100 as per manufacturer's instructions. Average response from at least duplicate tests is shown. Red numbers indicate a positive result.

Organism	Spike	Response (mV)	Result
B. megaterium ATCC 7703	$1x10^6$	36	True Negative
B. subtilis	$1x10^6$	23	True Negative
B. thuringiensis	$1x10^6$	37	True Negative
B. thuringiensis kurstakii (Dipel™)	0.5mg / mL	29	True Negative
Brucella abortus (ATCC 27565)	$6x10^6$	19	True Negative
B. canis (ATCC 23365)	$6x10^6$	14	True Negative
B. melitensis (ATCC 23456)	$6x10^6$	16	True Negative
B. suis (ATCC 23444)	$6x10^6$	18	True Negative
Burkholderia pseudomallei	$6x10^6$	5	True Negative
Clostridium botulinum Serotype-A	$6x10^6$	26	True Negative
C. botulinum Serotype-B	$6x10^6$	26	True Negative
C. botulinum Serotype-E	$6x10^6$	24	True Negative
C. botulinum Serotype-F	$6x10^6$	16	True Negative

Francisella tularensis LVS (ATCC 6223)	$6x10^6$	12	True Negative
F. tularensis - NYC isolate	$6x10^6$	8	True Negative
Bacillus cereus (ATCC 14579)	$1x10^6$	462	False Positive
B. cereus (ATCC 7064)	$1x10^6$	380	False Positive
B. cereus (Food poisoning Strain)	$1x10^6$	430	False Positive
B. cereus (Clinical Sample I)	$2x10^6$	178	False Positive
B. cereus (Clinical Sample II)	$1x10^6$	438	False Positive
Yersinia pestis (ATCC 11953)	$6x10^6$	11	True Negative

Conclusions and Recommendations

The QTL *B. anthracis* cartridge showed excellent discrimination against other potential biothreats and some near neighbors. Various *Bacillus cereus* spore preparations generated strong positive results therefore additional specificity testing to evaluate level of cross-reactivity was conducted (Table 2).

Table 2. Dilutions of *B. cereus* spore preparations which yielded positive readings on the *B. anthracis* cartridges (Table 2) were analyzed further. A positive result was defined as Response (mV) greater than 100 as per manufacturer's instructions. Red numbers indicate a positive result. ND = not done.

B. cereus (ATCC 14579)

	$1x10^6$	$5x10^5$	$2.5x10^5$	$1x10^5$	$1x10^4$
Sample #1	503	470	98	34	24
Sample #2	507	ND	160	ND	ND

B. cereus (ATCC 7064)

	$1x10^6$	$5x10^5$	$2.5x10^5$	$1.25x10^5$	$1x10^5$
Sample #1	354	167	142	125	97
Sample #2	406	218	175	91	90

B. cereus (Food Poisoning Strain)

	$1x10^6$	$5x10^5$	$2.5x10^5$	$1.25x10^5$	$1x10^5$
Sample #1	442	162	73	45	53
Sample #2	418	182	124	38	55

B. cereus (Clinical Sample I)

	$2x10^6$	$1x10^6$	$1x10^5$
Sample #1	159	34	30
Sample #2	197	50	30

B. cereus (Clinical Sample II)

	$1x10^6$	$1x10^5$	$1x10^4$	$1x10^3$	$1x10^2$	$1x10^1$
Sample #1	376	1222	1843	1190	253	36
Sample #2	500	1088	2308	1846	132	29

Conclusions and Recommendations

Four of five *B. cereus* strains generated negative results at or below $1x10^5$ spores per test suggesting either 1) low-level cross reactivity of *B. anthracis* cartridge with these strains or 2) possible false positive results due to spore clumping. One strain (a Wadsworth Center clinical isolate) generated strong positive signal down to 100 spores per cartridge. Negative control cartridges were run on the samples to determine whether the positive signals could be definitively attributed to antibody cross-reactivity or spore clumping (Table 3).

Table 3. Negative Control cartridge analysis of *B. cereus* strains which yielded false positive *B. anthracis* results. All *B. cereus* strains were confirmed negative for 3 gene targets in *B. anthracis* (one chromosomal assay, one pX01 assay, and one pX02 assay) by real-time PCR to confirm no culture or DNA contamination. Average Response (mV) from duplicate tests is shown. Red numbers indicate a positive result.

Organism	Spike	Response (mV)	Result
Bacillus anthracis Pasteur *(vegetative)*	$6x10^6$	12	True Negative
Bacillus anthracis Pasteur *(spores)*	$6x10^6$	23	True Negative
Bacillus cereus (ATCC 14579)	$1x10^6$	8	True Negative
B. cereus (ATCC 7064)	$1x10^6$	17	True Negative
B. cereus (Food poisoning strain)	$1x10^6$	16	True Negative
B. cereus (Clinical Sample)	$2x10^6$	18	True Negative
B. cereus (Clinical Sample)	$1x10^6$	229	False Positive
B. cereus (Clinical Sample)	$1x10^5$	366	False Positive
B. cereus (Clinical Sample)	$1x10^4$	144	False Positive
B. cereus (Clinical Sample)	$1x10^3$	19	True Negative

Conclusions and Recommendations

The data from evaluation using the Negative Control cartridges indicates *B. cereus* (Wadsworth Center clinical isolate 2) strain exhibits a high degree of clumping which causes false positive detection with the QTL detection system. Inclusion of the Negative Control cartridges into the QTL field protocol is essential for assay quality control

II- Sensitivity

Table 4. Serial dilutions of *B. anthracis* strains to determine limit of detection. A positive result was defined as Response (mV) greater than 100 as per manufacturer's instructions. Red numbers indicate a positive result. ND = not done.

Avirulent *B. anthracis* Sterne (pX02-) strain

	$1x10^6$	$1x10^5$	$1x10^4$	$1x10^3$	$5x10^2$	$2.5x10^2$	$1x10^2$
Test #1	2896	3031	1967	228	219	87	66
Test #2	2861	2898	1385	209	139	65	51

Avirulent *B. anthracis* Cohn (pX02-) strain

	$1x10^6$	$1x10^5$	$1x10^4$	$1x10^3$	$5x10^2$	$2.5x10^2$	$1x10^2$
Test #1	2656	2971	2488	214	115	94	25

Test #2	2533	3020	ND	142	125	107	27

Avirulent *B. anthracis* Pasteur (pX01-) strain

	$1x10^6$	$1x10^5$	$1x10^4$	$5x10^3$	$2.5x10^3$	$1.25x10^3$	$1x10^3$
Test #1	2976	1932	749	289	102	120	70
Test #2	899	2506	620	488	334	61	60

Virulent *B. anthracis* Ames strain (Brentwood)

	$1x10^6$	$1x10^5$	$1x10^4$	$5x10^3$	$2.5x10^3$	$1.25x10^3$	$1x10^3$
Test #1	2673	1505	672	351	161	90	59
Test #2	1680	1387	574	511	70	89	68

Conclusions and Recommendations

All strains of *B. anthracis* tested were detected consistently above 5000 spores, below the reported lethal dose of 7000 spores for this organism. Strain or spore preparation differences may account for 10 fold better sensitivity (LOD of 500 spores) seen in the Cohn and Sterne strain spore preparations. Interestingly, both of these strains lack the pX02 plasmid. Recommend establishing consistent LOD of 5000 organism but indicate in materials that detection below 1000 spores is common.

III: Robustness (matrix inhibition)

Assay robustness (Table 5) was evaluated by spiking Pasteur spores (which most closely matched the virulent Ames spores in QTL reader Response) at approximately 1 LOD (~5000 spores) into 0.1mg/mL of various powder matrices. These powders are a standard panel available from the US Army Critical Reagent Program for use in validation of assays for anthrax detection.

Table 5: Matrix effects on *B. anthracis* detection. 0.1mg/mL of the indicated powders were mixed with 5000 Pasteur spores and analyzed on the *B. anthracis* specific cartridges. A positive result was defined as Response (mV) greater than 100 as per manufacturer's instructions. Average Response from at least duplicate tests is shown. Red numbers indicate a positive result. The range of Responses from different Pasteur strain analyses are shown as a metric for Response variability above LOD.

Added Powder (0.1mg/mL)	Response (mV)	Result
None (neat Pasteur spike)	312 - 573	True Positive
Spackling Powder	179	True Positive
Baking Soda (Arm & Hammer)	934	True Positive
Instant Non-fat Dried Milk (Nestle Carnation)	652	True Positive
Talcum Powder (Equate)	453	True Positive
Flour (Gold Medal)	499	True Positive
Salt (Morton)	550	True Positive
Yeast (Red Star)	609	True Positive
Powdered Sugar (Confectioners)	294	True Positive
Dipel™ (Abbot Laboratories)	238	True Positive
Chalk (Bison)	272	True Positive

Foot Powder (Total Body)	522	True Positive
Ajax™ with Bleach (Colgate-Palmolive)	213	True Positive
Non-fat Dairy Creamer (Great Value)	520	True Positive
Kaolin (Fisher-Scientific)	528	True Positive
Bentonite (Aldrich)	356	True Positive
Aerosil R812S™ (Degussa)	485	True Positive

Conclusions and Recommendations
All strains of *B. anthracis* tested were detected consistently at 5000 spores, regardless of matrix material spiked. Baking soda and milk proteins increased response readings at LOD and might indicate paths to improving reader Response for *B. anthracis* detection.

IV. Post-VHP Operations Evaluation

Table 6 *Bacillus anthracis* Pasteur spores were examined Pre- and Post-VHP decontamination of the QTL reader. A positive result was defined as Response (mV) greater than 100 as per manufacturer's instructions. Red numbers indicate a positive Result. Asterisks (*) on the Average indicate mixed positive and negative results.

	Avirulent *B. anthracis* Pasteur (pX01-) strain						
Pre-VHP	$1x10^6$	$1x10^5$	$1x10^4$	$5x10^3$	$2.5x10^3$	$1.25x10^3$	$1x10^3$
Test #1	2976	1932	749	289	102	120	70
Test #2	899	2506	620	488	334	61	60
Average	1938	2219	685	389	218	91*	65
Post-VHP							
Test #1	266	2956	1595	1178	750	398	440
Test #2	1023	2693	2362	1495	675	124	94
Test #3	ND	ND	ND	ND	421	58	ND
Average	645	2825	1979	1337	365	192*	267*

Conclusions and Recommendations
There was no indication that the reader was significantly affected by the vapor hydrogen peroxide decontamination cycle. VHP decontamination of readers can be recommended.

Overall Conclusion
The consistent limit of detection of 2,500 to 5,000 *B. anthracis* spores, regardless of strain, is recommended. Outside of the first 3 orders of magnitude there is significant variability in reader Response (mV) which makes the results non-quantitative at extremely high quantities of spores. The threshold of 100 mV makes it easy for first responders to determine presence or absence of significant amounts of *B. anthracis*. Common hoax materials like talcum powder or dry milk should not interfere with the system and specificity with other organisms showed good selectivity. The time-to-result is comparable to other hand-held analytical tests though more hands on time is necessary.

Additional Recommendations

QA/QC: The false positive results with the one isolate of *B. cereus* suggest that the negative control cartridge (NCC) should be run prior to the target analyte cartridge, (TSC), *B. anthracis* in this case. This is for two reasons; 1) if the TSC is run first, the field response will escalate and the positive Response on the NCC will likely be misinterpreted as a confirmed positive, and 2) if the material is run on the NCC first and a positive result is obtained, that indicates field testing with the QTL system should not be performed. In addition, with the NCC run first, the user will have a chance to familiarize themselves with the protocol, and if that comes up positive, he/she should send the sample directly for testing and not run the target specific cartridge. If negative, then he/she can do the rule-in test with the TSC and only if positive, send to lab for confirmatory testing. We would recommend sending a positive control cartridge (PCC) along with the kit to confirm the reader is working appropriately prior to any sample analysis. This cartridge can and should be run before any other tests are performed in order to establish operational proficiency of both processor and QTL reader.

Test Cartridges: If in Level-A HazMat gear, it is difficult to hear the mixing ball move. This is a problem because the reader will give a positive result if the solution does not flow into the sample chamber. In addition, if in Level-A gear, the small syringe is difficult to manipulate and underfilling/overfilling sample cartridge is possible. Suggests incorporating one-volume syringes (i.e., pull completely to fill, push completely to discharge) in kits. While hand-shaking is satisfactory, a cartridge shaker would improve reproducibility and ease of use.

Reader: If recommended PPE is Level-A HazMat, buttons should be spaced further apart and syringes should be larger. Include reader operations and interpretation on inside lid of reader for field use.

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