Evaluation of a Rapid Immunoassay System for the Detection of *Bacillus anthracis* Spores

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Summary

- **RAMP® Anthrax Test is highly sensitive: 1,000 to 2,000 spores**
- **RAMP Anthrax Test does not cross react with non-anthracis bacilli strains**
- **None of the interferants tested caused false positive test results and the sensitivity of the test was maintained in the presence of interferants.**

Introduction

The RAMP Anthrax Assay is a product of Response Biomedical Corporation (Vancouver, British Columbia). The system consists of a RAMP reader, anthrax assay cartridges, sterile swabs, tips, pipettors and vials of sample buffer. The cartridge is a lateral flow immunoassay device with a test and control line. The detector is an antigen specific antibody attached to a fluorescent bead. The RAMP instrument detects the presence of fluorescent beads that attach to the capture assay line in the presence of specific antigen. The control line binds to excess beads passing across the line. The RAMP reader compares the intensity of the test line to the control line to make a positive/negative determination.

A sample is collected with the swab, placed into the sample buffer and expressed along the side of the vial. A tip containing the detector dried inside the wall of the tip is placed onto the pipettor and the sample is drawn into the pipettor. It is then gently pipetted in and out of the tip ten times to completely release the detector from the wall of the tip and mix with the sample. The sample is aspirated into the tip and pipetted into the sample well of the cartridge. The cartridge is then loaded into the RAMP reader. After a 15 minute incubation time, the result of the test is displayed on the screen of the RAMP reader.

Experimental Protocol

In this study, three non-pathogenic strains of *Bacillus anthracis* and three non-anthracis Bacilli strains were tested with the RAMP system. Bacteria were grown on sporulation agar to confluency, harvested, and washed three times with cold sterile water. The concentration of the spores was determined by plate count. Two-fold serial dilutions of anthracis strains were prepared in concentrations from 3.2×10^6 CFU/mL to 1.0×10^5 CFU/mL. Non-anthracis strains were prepared in concentrations of 10^8, 10^7 and 10^6 CFU/mL. In addition, 1.0 gram each of baking powder, flour and corn starch were suspended in 10 ml of sterile water to be tested as interferants. A 1 mL aliquot of each interferant suspension was seeded with 2×10^5 spores of *Bacillus anthracis* to determine the effect of interferant on test sensitivity.

In lieu of using the swab for collecting sample, 50 µL of sample buffer was removed from the sample vial and 10 µL of bacterial or interferant suspension pipetted into the vial. This mimics the amount of liquid retained by the swab and the amount of sample returned to the vial when using the swab to collect sample. The enclosed pipettor and tip was then used to aspirate the sample containing suspension from the sample vial and into the sample well of the cartridge. Samples were run in duplicate on each of two machines, each having a different operator in order to test both instrument and operator reproducibility. If there was one positive and one negative result, an additional test was performed. Two concordant tests determined the final result.

Results

Table 1: Sensitivity and Specificity

Organism	Strain	Concentration (cfu/ml)	# Of Spores per Test	Instrument	Result
Bacillus anthracis	NNR-1	1.00E + 05	1.00E + 03	1	NEG
		1.00E + 05	1.00E + 03	2	NEG
		2.00E + 05	2.00E + 03	1	POS
		2.00E + 05	2.00E + 03	2	POS
		4.00E + 05	4.00E + 03	1	POS
		4.00E + 05	4.00E + 03	2	POS
		8.00E + 05	8.00E + 03	1	POS
		8.00E + 05	8.00E + 03	2	POS
		1.60E + 06	1.60E + 04	1	POS
		1.60E + 06	1.60E + 04	2	POS
		3.20E + 06	3.20E + 04	1	POS
		3.20E + 06	3.20E + 04	2	POS
Bacillus anthracis	d-Sterne	1.00E + 05	1.00E + 03	1	NEG*
		1.00E + 05	1.00E + 03	2	POS
		2.00E + 05	2.00E + 03	1	POS
		2.00E + 05	2.00E + 03	2	POS
		4.00E + 05	4.00E + 03	1	POS
		4.00E + 05	4.00E + 03	2	POS
		8.00E + 05	8.00E + 03	1	POS
		8.00E + 05	8.00E + 03	2	POS
		1.60E + 06	1.60E + 04	1	POS
		1.60E + 06	1.60E + 04	2	POS
		3.20E + 06	3.20E + 04	1	POS
		3.20E + 06	3.20E + 04	2	POS
Bacillus anthracis	d-Ames	1.00E + 05	1.00E + 03	1	POS
		1.00E + 05	1.00E + 03	2	POS
		2.00E + 05	2.00E + 03	1	POS
		2.00E + 05	2.00E + 03	2	POS
		4.00E + 05	4.00E + 03	1	POS
		4.00E + 05	4.00E + 03	2	POS
		8.00E + 05	8.00E + 03	1	POS
		8.00E + 05	8.00E + 03	2	POS
		1.60E + 06	1.60E + 04	1	POS
		1.60E + 06	1.60E + 04	2	POS
		3.20E + 06	3.20E + 04	1	POS
		3.20E + 06	3.20E + 04	2	POS
Bacillus subtilis	var. globigii	1.00E + 06	1.00E + 04	1	NEG
		1.00E + 07	1.00E + 05	1	NEG
		1.00E + 08	1.00E + 06	1	NEG
Bacillus thuringiensis	"kustaki"	1.00E + 06	1.00E + 04	1	NEG
		1.00E + 07	1.00E + 05	1	NEG
		1.00E + 08	1.00E + 06	1	NEG
Bacillus cereus		1.00E + 06	1.00E + 04	1	POS * *
Bacillus cereus	6E1	1.00E + 06	1.00E + 04	1	NEG
	1219	1.00E + 06	1.00E + 04	1	NEG

* This result was designated as negative since 2 of 3 tests were negative. All other results represent identical duplicate tests.

** The strain of Bacillus cereus (6E1) originally used for testing showed a positive result. It was later determined that the culture was contaminated.

Table 2: Interferants

Interferant	Concentration (cfu/ml)	# Of Spores per test	Instrument	Result
Baking powder	0	0	1	NEG
	0	0	2	NEG
	2.00E + 05	2.00E + 03	1	POS
	2.00E + 05	2.00E + 03	2	POS
Flour	0	0	1	NEG
	0	0	2	NEG
	2.00E + 05	2.00E + 03	1	POS
	2.00E + 05	2.00E + 03	2	POS
Corn starch	0	0	1	NEG
	0	0	2	NEG
	2.00E + 05	2.00E + 03	1	POS
	2.00E + 05	2.00E + 03	1	POS

Discussion

The anthrax assay limit of detection for the NNR-1 and delta Sterne strains was 2000 spores and 1000 spores for the delta Ames strain. The strain of *Bacillus cereus* (6E1) originally used for testing showed a positive result. It was later determined that the culture was contaminated. A different strain of *B. cereus* (1219) was tested at 1 x 10^6 and found not to cross-react. None of the three interferants caused false positive test results and the sensitivity of the test was maintained in the presence of the interferants (2,000 spores for the delta Ames strain).