The RAMP® Anthrax Test

A Reliable, Sensitive and Rapid System for Detecting *Bacillus anthracis* Spores

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Results from three external studies confirm in-house data demonstrating that Response Biomedical Corp.'s RAMP Anthrax Test is able to rapidly and reliably detect *Bacillus anthracis* (*B. anthracis)* in samples containing 4000 or more spores. Anthrax is a serious infectious disease caused by *B. anthracis*, the gram-positive spore-forming bacilli. It has been estimated that the dose required to produce an infection in 50% of hosts (ID_{50}) is approximately 10,000 *B. anthracis* spores[1]. Early identification of the infectious agent is a key factor in preventing serious outcomes.

RAMP is a platform technology that can be adapted to quantify virtually any immunologically active substance. The RAMP System consists of two components: a disposable Test Cartridge that houses an analyte-specific immunochromatographic strip, and a portable fluorescence Reader that is used to quantify antibody-antigen complexes. The membrane strip component uses latex particles that are fluorescently labeled and tagged with antibodies. Immunoassays based on fluorescence have substantially greater sensitivity and dynamic range than those based on earlier-generation detection techniques such as reflectance. A proprietary Internal Control runs concurrently in every assay, allowing the RAMP System to compensate for test-to-test variability.

The RAMP Reader has received clearance from the U.S. FDA as a Class II medical device and a Medical Device License from the Canadian Therapeutic Products Directorate.

The RAMP System is well suited to perform environmental tests for detection of infectious agents, such as *B. anthracis*. A suspect surface is swabbed or powder collected and then added to the Sample Buffer. An aliquot of the sample is placed into the Test Cartridge that is then inserted into the RAMP Reader. The Reader measures the amount of fluorescence emitted from the Test Cartridge, and the test result is displayed as

[1] www.bt.cdc.gov/Agent/Anthrax

POSITIVE or NEGATIVE in less than 15 minutes. This result can be stored, printed or uploaded to a computer system as needed.

Performance of the RAMP Anthrax Test has been evaluated at three external sites: the Maryland State Department of Health (March, 2002); Seattle-based Intertox, Inc. (July, 2002); and DRDC Suffield, a research establishment within the Canadian Department of National Defence (July, 2002). Data from these three evaluations support the claims of a high level of sensitivity and specificity for the RAMP System.

Maryland State Department of Health

The first external evaluation was conducted at the Maryland State Department of Health. Sensitivity of the RAMP Test for detection of *B. anthracis* (BA) spores was evaluated by testing serial dilutions of avirulent spores (Pasteur) prepared in water. Aliquots (10 µL) of each suspension were pipetted directly onto swabs. Spores were recovered into RAMP Sample Buffer, delivered into RAMP Test Cartridges and assayed in the RAMP Reader. Results from all samples containing 4000 spores/swab and greater were reported as positive (Table 1). In 8/10 samples that contained 2000-3,300 spores/swab the test results were also positive, while test results from all samples containing 1000 spores/swab were negative. Testing in triplicate of aliquots of a 0.5 McFarland culture of vegetative *B. anthracis* cells also showed positive results at 10^6 Colony Forming Units (CFU)/swab.

Table 1. RAMP Anthrax Sensitivity Test Results from Maryland State Department of Health

Sample	CFU/Swab	Test Result Pos/Total
BA spores	10^6	3/3
BA spores	10^5	3/3
BA spores	10^4	4/4
BA spores	$8x10^3$	3/3
BA spores	$6x10^3$	3/3
BA spores	$5x10^3$	7/7
BA spores	$4x10^3$	3/3
BA spores	$3.3x10^3$	3/4
BA spores	$3x10^3$	3/3
BA spores	$2x10^3$	2/3
BA spores	10^3	0/3
BA vegetative cells	10^6	3/3

Suspensions of each of five different powders were prepared and aliquots were assessed in triplicate for interference to the RAMP test. Test results for all 15 samples were negative (Table 2). Suspensions of each powder were then spiked with known concentrations of *B. anthracis* and tested in RAMP. Test results from all samples containing 10^5 and 10^4 *B. anthracis* spores/swab were positive and test results from 14/15 samples containing 1000 *B. anthracis* spores/swab were negative (Table 2). These results show that the five powders did not exhibit any positive or negative interference with RAMP assay at the levels tested.

Further specificity testing included *Bacillus cereus* (BC) *Bacillus subtilus* (BS) spores (up to 10^6 spores) tested alone or as above, in the presence of each of the five different powders. All test results were negative (Table 2). All test results from 10^6 CFU/swab vegetative cells of BS or BC were also negative. No false positive results

were reported, resulting in 100% specificity of the RAMP Anthrax Assay (Table 2).

Table 2. RAMP Anthrax Specificity and Interfering Substance Test Results from Maryland State Department of Health

Sample	CFU/ Swab	Test Result Pos/Total
Powders	0	0/15
BA spores in powder	10^5	15/15
BA spores in powder	10^4	15/15
BA spores in powder	10^3	1/15
BC spores	10^6	0/3
BC spores	10^5	0/3
BC spores in powder	10^5	0/15
BC spores in powder	10^4	0/15
BS spores	10^6	0/3
BS spores	10^5	0/3
BS spores in powder	10^5	0/15
BS spores in powder	10^4	0/15
BC vegetative cells	10^6	0/3
BS vegetative cells	10^6	0/3

Intertox, Inc., Seattle, Washington

A second evaluation was conducted at Intertox, Inc., an independent, Seattle-based public and occupational health firm. Sensitivity of the RAMP Test for detection of *B. anthracis* spores was evaluated by testing serial dilutions of avirulent *B. anthracis* spores (Sterne Vaccine) prepared in water. Aliquots (10 µL) of each suspension were pipetted directly into the Sample Buffer. Aliquots were delivered into RAMP Test Cartridges and assayed in the RAMP Reader. Results from all samples containing 1600 spores/vial and greater were positive (Table 3). Samples that contained 800 spores or less were negative. Limited specificity testing showed that results from samples containing *Bacillus thuringiensis* (BT) at 10^6 spores were negative.

Table 3. RAMP Anthrax Sensitivity Test Results from Intertox, Inc.

Sample	CFU/ Vial	Test result Positive/Total
BA spores	6.4×10^4	1/1
BA spores	3.2×10^4	1/1
BA spores	6.4×10^3	1/1
BA spores	3.2×10^3	3/3
BA spores	1.6×10^3	3/3
BA spores	8.0×10^2	0/3
Water	0	0/3
BT spores	10^6	0/3

DRDC Suffield, Canada

The third evaluation was conducted at DRDC Suffield, part of Defence R&D Canada, an agency of the Canadian Department of National Defence[2]. Sensitivity of the RAMP Assay for detection of *B. anthracis* spores was evaluated by testing serial dilutions of virulent spores. These were done in two sets (Ames strain crude or Ames strain washed and heat shocked) prepared in water and plated to confirm CFU/mL. Aliquots (10 µL) of each suspension were pipetted directly into the Sample Buffer. Aliquots of the Sample Buffer were then delivered into RAMP Test Cartridges and assayed in the RAMP Reader. Results from all crude samples containing 750 CFU/vial and greater were reported as positive (Table 4). Samples that contained 380 CFU/vial or less were negative. Results from all heat shocked, washed samples containing 3100 CFU/vial and greater were reported as

[2] While the results presented here are very promising, and noting that rigid experimental protocols were observed, DRDC Suffield considers these results preliminary. DRDC Suffield is planning additional studies to extend and confirm preliminary findings with the RAMP® technology.

positive, samples that contained 1600 spores tested positive in 2/3 replicates and samples containing 800 spores or less were negative.

Table 4. RAMP Anthrax Test Sensitivity Results from DRDC Suffield

Sample	CFU/Vial	Test Result Positive/Total
BA, Ames (crude, not heat shocked, plated)	$1.5x10^6$	1/1
	$1.5x10^5$	1/1
	$1.5x10^4$	1/1
	$1.5x10^3$	1/1
	$7.5x10^2$	3/3
	$3.8x10^2$	0/1
	$1.9x10^2$	0/1
	$1.5x10^2$	0/1
	$1.5x10^2$	0/1
BA, Ames (washed, heat shocked, plated)	$2.5x10^5$	1/1
	$2.5x10^4$	1/1
	$1.25x10^4$	1/1
	$6.3x10^3$	1/1
	$3.1x10^3$	1/1
	$1.6x10^3$	2/3
	$8.0x10^2$	0/1
	$2.5x10^2$	0/1

Limited specificity testing (Table 5) showed that results from samples containing *Bacillus thuringiensis* (BT) or *Bacillus globigii* (BG) spores at 10^7 CFU or less were negative. Cross reactivity to BG spores was observed at $2x10^8$ CFU, a concentration at least 50,000 times the *B. anthracis* detection level resulting in cross reactivity of .002%. It is important to note that the internal timer in the Reader used in this study was bypassed to allow greater sample throughput. This allowed the BG tests performed at $2x10^8$ to continue until the test was reported as positive. Ordinarily, with the timer activated, this high concentration of BG would have resulted in a reader message that the system was overloaded directing the user to dilute the sample. A similar level of BT spores ($1.5x10^8$) clogged the sample pad and gave no test result. Again, with the timer functioning, the reader message would be displayed.

Table 5. RAMP Anthrax Specificity Test Results from DRDC Suffield

Sample	CFU/Vial	Test Result Positive/Total
BG	$2x10^8$	6/6
	$2x10^7$	0/4
	$6.2x10^6$	0/3
BT	$1.5x10^8$	-/3
	$1.5x10^7$	0/3
	$1.5x10^6$	0/3

Conclusion

Results from three external studies confirm in house data demonstrating that the RAMP Anthrax Test was able to reliably report a POSITIVE result for all samples containing 4000 or greater *B. anthracis* spores. In two studies the sensitivity was higher, 750 spores at DRDC Suffield and 1600 spores at Intertox. No false positive results were observed in any of the studies from any sample that contained less than 10^7 spores of a related *Bacillus* strain.