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Fluorogenic Hand-held Immunoassay for Detection and Identification of Anthrax:

Preliminary Evaluation of the Rapid Analyte Measurement Platform

R.E. Fulton, N.D. Barab, L.L. Stadnyk, and L.M. Negrych

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Abstract

The Rapid Analyte Measurement Platform (RAMP™) hand-held assay (HHA) system was evaluated for sensitivity and specificity in detection of *B. anthracis* by challenging with killed and live *B. anthracis* and closely related *Bacillus* strains in the BSL2 and BSL3 laboratories at DRDC Suffield. The limit of detection (LOD) of RAMP™ HHAs for detection of killed *B. anthracis* was 2.6 x 10E6 cfu/mL, corresponding to 2.6 x 10E4 cfu per test. Live *B. anthracis* (washed, heat shocked) and crude *B. anthracis* (not washed or heat shocked) were detected at 3.1 x 10E5 cfu/mL and 7.5 x 10E4 cfu/mL, respectively; these values correspond to a LOD per test of 3.1 x 10E3 cfu and 7.5 x 10E2 cfu, respectively. RAMP™ anthrax HHAs did not cross-react with other, closely related *Bacillus* strains at the concentrations examined, with the exception of *B. globigii*, which cross-reacted at a concentration in excess of 2.0 x 10E9 cfu/mL. The RAMP™ system may be a valuable alternative to chromogenic HHAs for rapid, on-site detection of *B. anthracis* by First Responders and military personnel.

R□sum□

Le syst□me manuel de biotest (HHA) de Plaques de mesure rapide d□analyte (RAMP™) a □t□ □valu□ pour sa sensibilit□ et sp□cificit□ dans la d□tection de *B. anthracis* en le mettant □l□preuve avec des *B. anthracis* vivants et morts et des souches de *B. anthracis* □troitement reli□es, dans les laboratoires de bios□curit□ de niveau 2 et 3 □RDDC Suffield. La limite de d□tection du syst□me HHA RAMP™ pour la d□tection de *B. anthracis* mort □tait de 2.6 x 10E6 cfu/mL, correspondant □2.6 x 10E4 cfu par essai. *B. anthracis* vivant (lav□ et soumis au choc thermique) et *B. anthracis* (non lav□ ni soumis au choc thermique) ont □t□ d□tect□s □3.1 x 10E5 cfu/mL et 7.5 x 10E4 cfu/mL respectivement; ces valeurs correspondent □une limite de d□tection de 3.1 x 10E3 cfu et 7.5 x 10E2 cfu respectivement. Le syst□me HHA RAMP™ de charbon bact□ridien n□a pas provoqu□ de r□action antig□nique crois□e avec d□autres souches *Bacillus* □troitement reli□es, aux concentrations examin□es, avec l□exception du *B. globigii*, qui a eu une r□action antig□nique crois□e □une concentration sup□rieure □2.0 x 10E9 cfu/mL. Le syst□me RAMP™ peut □tre une alternative valable au syst□me HHA chromog□nique pour une d□tection rapide, sur place de *B. anthracis* par les premiers intervenants et le personnel militaire.

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Executive summary

Fluorogenic Hand-held Immunoassay for Detection and Identification of Anthrax: Preliminary Evaluation of the Rapid Analyte Measurement Platform

R.E. Fulton; N.D. Barab□; L.L. Stadnyk; L.M. Negrych; DRDC Suffield TM 2006-250; Defence R&D Canada □ Suffield; December 2006.

Background: Hand-held immunoassays (HHAs) are rapid-response, disposable devices based on a lateral flow, immunochromatographic chemistry that relies on the binding properties of antibodies for specific antigens. HHAs have application for use by military personnel and First Responders, for on-site detection and identification of biological threat agents. DRDC Suffield has evaluated a number of HHA technologies, the majority of which utilized a chromogenic reporter system and required visual inspection for reading and analysis of results. More recently, DRDC Suffield evaluated a fluorogenic HHA system, the Rapid Analyte Measurement Platform (RAMP^{TM}), a proprietary technology developed by Response Biomedical Corp. (Burnaby, BC). This technology utilized a fluorescence reporter system and a portable fluorimeter and offered the advantages of enhanced sensitivity (due to the use of a fluorogenic versus chromogenic reporter system), as well as unbiased, qualitative recording of results. In June 2002, a preliminary laboratory evaluation of the RAMP^{TM} system for detection and identification of anthrax was conducted in the BSL2 and BSL 3 laboratories at DRDC Suffield. Subsequent to this preliminary work, the RAMP^{TM} system was validated by the Association of Analytical Communities (AOAC) International and was the only hand-held device to receive AOAC certification for detection of anthrax. The RAMP^{TM} system has also been evaluated, following AOAC guidelines as set out for anthrax, for another biothreat agent, ricin.

Results: In the preliminary studies described in this report, the RAMP^{TM} system was evaluated for sensitivity and specificity in detection of *B. anthracis* by challenging with killed and live *B. anthracis* and closely related *Bacillus* strains in the BSL2 and BSL3 laboratories at DRDC Suffield. The LOD of RAMP^{TM} HHAs for detection of killed *B. anthracis* was 2.6 x 10E6 cfu/mL, corresponding to 2.6 x 10E4 cfu per test. Live *B. anthracis* (washed, heat shocked) and crude *B. anthracis* (not washed or heat shocked) were detected at 3.1 x 10E5 cfu/mL and 7.5 x 10E4 cfu/mL, respectively; these values correspond to a LOD per test of 3.1 x 10E3 cfu and 7.5 x 10E2 cfu, respectively. The RAMP^{TM} system did not cross-react with other closely related *Bacillus* strains at the concentrations examined, with the exception of *B. globigii*, which cross-reacted at a concentration in excess of 2.0 x 10E9 cfu/mL.

Significance: This preliminary study suggests that the RAMP^{TM} system offers enhanced sensitivity over conventional chromogenic HHAs for identification of *B. anthracis*. Based on the results of this present study and subsequent studies that have been conducted to evaluate the RAMP^{TM} system, the RAMP^{TM} is suggested as a valuable alternative to chromogenic HHAs for rapid, on-site detection of *B. anthracis* by First Responders and military personnel. At the time of writing, the RAMP^{TM} system has been adopted for use by the Royal Canadian Mounted Police and First Responders for several Canadian cities and police departments (B. Radvac, personal communication).

Future plans: A more comprehensive evaluation of the RAMP™ system for detection of *B. anthracis* in a variety of types of environmental sample matrices is warranted. The evaluation of the RAMP™ system for efficacy in detection of other high priority biological threat agents is also recommended.

Sommaire

Fluorogenic Hand-held Immunoassay for Detection and Identification of Anthrax: Preliminary Evaluation of the Rapid Analyte Measurement Platform

R.E. Fulton; N.D. Barab ; L.L. Stadnyk; L.M. Negrych; DRDC Suffield TM 2006-250; R & D pour la d fense Canada Suffield; d cembre 2006.

Contexte: Les immuno-essais manuels (HHA) sont des appareils de r ponse rapide jetables bas s sur une chimie immuno-chromatographique, de courant secondaire, qui fonctionne sur les propri t s de liaison des anticorps d antig nes sp cifiques. Ces immuno-essais peuvent tre utilis s par le personnel militaire et les premiers intervenants, pour la d tection et l identification sur place d agents repr sentant une menace biologique. RDDC Suffield a valu un certain nombre de technologies d immuno-essais manuels, la majorit desquels utilisaient un syst me chromog nique de communication des r sultats et exigeaient une inspection visuelle pour lire et analyser les r sultats. RDDC Suffield a valu plus r cemment, un syst me d immuno-essais manuel fluorog nique, la plaque de mesure rapide d analyte (RAMP^{TM}), une technique brevet e, mise au point par Response Biomedical Corp. (Burnaby, CB). Cette technologie utilisait un syst me de communication des r sultats fluorescence et un fluorim tre portable qui offrait l avantage d une sensibilit am lior e (due l utilisation d un syst me fluorog nique de communication des r sultats contrairement un syst me chromog nique), ainsi qu une documentation qualitative et impartiale des r sultats. En juin 2002, une valuation pr liminaire en laboratoire du syst me RAMP^{TM}, visant d tecter et identifier le charbon bact ridien, a t effectu e dans des laboratoires de conditions de bios curit de niveau 2 et 3, RDDC Suffield. Ult rieurement ces travaux pr liminaires, le syst me RAMP^{TM} avait t valid par l Association of Analytical Communities (AOAC) internationale et tait le seul appareil manuel avoir re u l homologation AOAC pour la d tection du charbon bact ridien. Le syst me RAMP^{TM} a aussi t valu , selon les principes directeurs AOAC tablis pour le charbon bact ridien, pour le ricin, un autre agent de bioterrorisme,

Les r sultats: Dans les tudes pr liminaires d crites dans ce rapport, le syst me RAMP^{TM} est valu pour sa sensitivit et sp cificit dans la d tection de *B. anthracis,* en le mettant l preuve avec des *B. anthracis* vivants et morts et des souches de *B. anthracis* troitement reli es, dans les laboratoires de bios curit de niveau 2 et 3 RDDC Suffield. La limite de d tection du syst me HHA RAMP^{TM} pour la d tection de *B. anthracis* mort tait de 2.6 x 10E6 cfu/mL, correspondant 2.6 x 10E4 cfu par essai. *B. anthracis* vivant (lav et soumis au choc thermique) et *B. anthracis* (non lav ni soumis au choc thermique) ont t d tect s 3.1 x 10E5 cfu/mL et 7.5 x 10E4 cfu/mL respectivement; ces valeurs correspondent une limite de d tection de 3.1 x 10E3 cfu et 7.5 x 10E2 cfu respectivement; Le syst me HHA RAMP^{TM} de charbon bact ridien n a pas provoqu de r action antig nique crois e avec d autres souches *Bacillus* troitement reli es aux concentrations examin es, avec l exception du *B. globigii*, qui a eu une r action antig nique crois e une concentration sup rieure 2.0 x 10E9 cfu/mL.

La portée des résultats : Cette étude préliminaire suggère que le système RAMP™ offre une sensitivité améliorée à la détection du *B. anthracis,* comparé aux HHA chromogéniques classiques. En se basant sur les résultats de la présente étude et des études ultérieures qui ont été conduites pour évaluer le système RAMP™, on suggère que le système RAMP™ est une alternative valable aux systèmes chromogéniques HHA pour une détection rapide, sur place, de *B. anthracis* par les premiers intervenants et le personnel militaire. À cette heure, le système RAMP™ a été adopté pour être utilisé par la Gendarmerie royale du Canada et par les premiers intervenants de plusieurs villes canadiennes et départements de police (B. Radvac, communication personnelle).

Plans futurs: Une évaluation plus approfondie du système RAMP™, pour la détection de *B. anthracis* dans une variété de types de matrices d'échantillons environnementaux est justifiée. On recommande aussi d'évaluer le système RAMP™ pour son efficacité à détecter d'autres agents de bioterrorisme de haute priorité.

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1 Introduction

Hand-held immunoassays (HHAs) for detection of antigen are rapid-response, disposable devices based on a lateral flow, immunochromatographic chemistry that relies on the binding properties of antibodies for specific antigens. HHAs hold a number of advantages over more traditional immunoassays, such as simplicity of operation, short reaction time, and relative low cost, thus enabling economic point-of-care use for clinical applications. HHAs also have application for use by military personnel and First Responders, for on-site detection and identification of biological threat agents. Preliminary identification of biological agents with these devices would allow for advance initiation of medical countermeasures until such time as confirmatory analysis could be conducted using additional identification technologies. HHAs have been described for the identification of a number of potential biological threat agents, including *Bacillus anthracis* [1, 2, 3], ricin [4], *Francisella tularensis* [5], *Burkholderia mallei* [6], Staphylcoccal enterotoxin B [7], *Brucella spp*. [8], *Yersinia pestis* [2], and aflatoxin [9]. A review of immunochromatographic assays for detection and identification of infectious diseases and biological warfare agents has also been published [10].

Our institution has, over the last decade, evaluated a number of HHA technologies based on lateral flow chemistries. The majority of these have utilized a chromogenic reporter system that relied upon visual inspection by the operator to read and analyze the results. More recently, DRDC Suffield evaluated another HHA system, the Rapid Analyte Measurement Platform (RAMP^{TM}), a proprietary technology developed by Response Biomedical Corp. (Burnaby, BC). This technology utilized a fluorescence reporter system and a portable fluorimeter and, hence, held the promise of being more sensitive in detection of analyte; as well it provided for unbiased analysis of results. In June 2002, a preliminary laboratory evaluation of the RAMP^{TM} system for detection and identification of anthrax was conducted in the BSL2 and BSL3 laboratories at DRDC Suffield. The results of that study are presented and discussed in this report. Findings indicated that the RAMP^{TM} HHA system detected killed *B. anthracis* at a limit of detection (LOD) of 2.6 x 10E6 cfu/mL, corresponding to 2.6 x 10E4 cfu per test. Live *B. anthracis* (washed, heat shocked) and crude *B. anthracis* (not washed, not heat shocked) were detected at 3.1 x 10E5 cfu/mL and 7.5 x 10E4 cfu/mL, respectively; these values correspond to a LOD per test of 3.1 x 10E3 cfu and 7.5 x 10E2 cfu, respectively. The RAMP^{TM} system did not cross-react with other closely related *Bacillus* strains at the concentrations examined, with the exception of *B. globigii*, which cross-reacted at a concentration in excess of 2.0 x 10E9 cfu/mL.

Since DRDC Suffield performed the preliminary evaluation of the RAMP^{TM} system for detection of anthrax, described in this Technical Memorandum, the RAMP^{TM} was further evaluated by the Association of Analytical Communities (AOAC) International (11), a US-based organization that develops performance standards for validation of biodetection devices (12). The RAMP^{TM} system for detection of anthrax assessed in this present study was the only hand-held device to meet AOAC performance standards and receive AOAC certification for detection of anthrax (13). The RAMP^{TM} system has also been evaluated, following AOAC guidelines as set out for anthrax, for another biothreat agent, ricin. These latter studies were conducted by DRDC Suffield in collaboration with US Edgewood Chemical and Biological Center, and the CBRN MOU Detection and Diagnostic Reagents Working Group, and are reported elsewhere (14).

2 Materials and Methods

2.1 The RAMP™ System

The RAMP™ system is comprised of a portable fluorescence reader and a disposable test cartridge enclosing a nitrocellulose immunochromatographic strip. Test sample is mixed with sample buffer and transferred to the cartridge sample well with a pipette tip that has been pre-infused with fluorescent-labeled latex beads coated with analyte-specific antibody (detector antibody). Analyte in the sample binds to the antibody-coated latex beads. The bead complex is transported through the strip by capillary action. At the detection zone, the complex is captured and arrested by a second analyte-specific antibody (capture antibody) that is embedded in the strip. Excess unbound latex beads migrate past the detection zone and are arrested at the control zone where they react with an anti-species antibody (control antibody) immobilized in the strip. After a specified incubation time, the reader scans the test strip for fluorescence through an opening in the bottom of the cartridge. A bar code on the test cartridge containing test-specific information is also read. The reader calculates the ratio of concentration of fluorescing beads at the detection and control zones and converts this figure to analyte concentration via an analyte-specific calibration curve. By calculating assay results as a ratio between two measurements, the RAMP™ system accounts for variation in sample and membrane properties. Cartridges are supplied together with lot cards which provide specific lot information, including lot number, expiration date, and standard concentration curve with positive/negative cut-off.

Diagrammatic representations of the operational procedures and mechanism of operation of the RAMP™ system are provided in Figures 1 and 2, respectively.

2.2 Buffers and Materials

Phosphate buffered saline (PBS) was purchased from Sigma-Aldrich Canada (Oakville, ON). Bovine serum albumin was purchased from Roche Diagnostics Canada (Laval, QC). RAMP™ buffer was a gift from Response Biomedical Corp.

A RAMP™ reader, RAMP™ anthrax cartridges, and RAMP™ anthrax lot cards were provided by Response Biomedical Corp.

2.3 Antigens

2.3.1 Inactivated: Gamma-irradiated *B. anthracis* vollum, washed, DPG 960104, 1.3x10E8 cfu/mL, and gamma-irradiated *B. thuringiensis*, Israel, DPG 22 Jan 99, 1.8x10E8 cfu/mL were gifts from US Dugway Proving Ground (DPG) (Utah, MT).

2.3.2 Live: *B. anthracis* Ames strain was from DRDC Suffield stocks (gift from Dr Dan Dragon, DRDC Suffield). *Bacillus subtilis var niger* (*Bacillus globigii*) was originally from DPG and stored as powder at DRDC Suffield; a 1% stock solution of *B. globigii* was prepared in PBS and stored at 4 °C until used. *Bacillus thuringiensis,* subspecies *kurstaki* was from Safer's BTK™ biological insecticide, 10,600 IU/mg and was purchased from a local greenhouse. A working

stock was prepared by dilution 1:100 in PBS, pH 7.4 (Sigma-Aldrich Canada). *B. cereus* 11778, obtained originally from the American Type Culture Collection (ATTC) (Manassa, VA), was provided as a gift by Dr Dan Dragon, DRDC Suffield.

Pure cultures of *B. anthracis* were grown by streaking an aliquot of stock culture onto 5% sheep blood agar plates. Individual colonies were picked and subcultured in Nutrient Broth (BD Diagnostics, Edmonton, AB). For experiments, *B. anthracis* was either washed and heat-shocked (to kill vegetative cells), or was used as a crude preparation (not washed, not heat shocked). To prepare washed and heat shocked bacteria, cells were washed five times in sterile saline by centrifugation (12,000g for 20 minutes) and resuspended in 1 mL sterile saline; cultures were then heat shocked for 20 minutes in a 65 ^{o}C water bath. *B. globigii*, *B. cereus*, and *B. thuringiensis* were similarly washed in sterile saline (10,000g for 10 minutes) and heat shocked (65 ^{o}C for 30 minutes). All bacterial preparations (*B. anthracis*, *B. thuringiensis*, *B. cereus*, and *B. globigii*) were enumerated prior to use by plating dilutions (10E4, 10E5, and 10E6) on Nutrient or blood agar plates, followed by overnight incubation at 37 ^{o}C, and counting of colonies (by eye). Final cell counts for *B. anthracis* (washed and heat shocked), *B. anthracis* (crude, not heat shocked) were 3.0 x 10E7 and 5.0 x 10E9 cfu/mL, respectively; cell counts for *B. globigii*, *B. thuringiensis*, and *B. cereus* (washed and heat shocked) were 2 x 10E10, 1.5 x 10E10, and 1.1 x 10E4 cfu/mL respectively.

2.4 Procedures

Procedures for performance of RAMP^{TM} assays were as suggested by Response Biomedical Corp. Serial 10-fold dilutions of bacteria were prepared in PBS. For each concentration to be tested, a 10 µL aliquot of bacterial suspension was transferred to a microfuge tube containing 90 µL of RAMP^{TM} sample buffer. Negative controls were prepared by adding 10 µL PBS to 90 µL RAMP sample buffer. The RAMP^{TM} cartridge and accompanying pipette tip were removed from the cartridge pouch. The pipette tip was fitted to a variable volume Gilson Pipetman pipettor (Mandel Scientific, Guelph, ON) and the sample was mixed thoroughly by pipetting up and down slowly 10 times. A 70 µL volume of the sample was delivered to the sample well and the reaction was allowed to develop (outside the reader) for 14 minutes before it was inserted into the reader and read. Additional samples were similarly processed and read at two minute intervals, thus allowing for the concurrent running of six consecutive tests within 14 minutes. Once inserted into the reader, the cartridge was automatically scanned and the result displayed on the reader screen as positive or negative. Beginning with the highest bacterial concentration, samples were typically read in single replicates until a positive reaction was obtained; at this point, in order to refine the assay LOD, serial two-fold dilutions were prepared and assays were performed and read in triplicate. The LOD was defined as the concentration at which three out of three assay replicates were positive.

Assays utilizing killed agents and live *B. thuringiensis* and *B. globigii* were performed in the BSL2 laboratory at DRDC Suffield; assays utilizing live *B. anthracis* were performed in the BSL3 containment laboratory at DRDC Suffield.

3 Results and Discussion

Results obtained by RAMP™ analysis of killed *B. anthracis* are provided in Table 1. The approximate LOD of the RAMP™ assay for detection of killed *B. anthracis* was 2.6 x 10E6 cfu/mL (2.6 x 10E4 cfu/test). *B. thuringiensis*, a closely related *Bacillus* strain, did not cross-react in the assay at 1.0 x 10E8 cfu/mL, the highest concentration tested.

Table 1. RAMP™ analysis of killed B. anthracis

Agent	Concentration (cfu/mL)	Positive/Total	Approximate LOD (cfu/mL)	Approximate LOD (cfu/test)
B. anthracis	2.6 x 10E6 1.3 x 10E6 6.5 x 10E5 3.2 x 10E5 1.6 x 10E5 8.0 x 10E4 3.8 x 10E4	3/3 1/3 0/1 0/1 0/1 0/1 0/1	2.6 x 10E6	2.6 x 10E4
B. thuringiensis	1.0 x 10E8 1.0 x 10E7 1.0 x 10E6	0/3 0/1 0/1	N/A*	N/A

* N/A: not applicable

RAMP™ analysis of live *B. anthracis* (crude, not heat shocked) and *B. anthracis* (washed, heat shocked) are provided in Table 2. The approximate LOD of the RAMP™ assay for detection of live *B. anthracis* was 7.5 x 10E4 cfu/mL (7.5 x 10E2 cfu/test) and 3.1 x 10E5 cfu/mL (3.1 x 10E3 cfu/test) for crude, non-heat shocked and washed, heat-shocked preparations, respectively.

Table 2. RAMP™ analysis of live B. anthracis

Agent	Concentration (cfu/mL)	Positive/Total	Approximate LOD (cfu/mL)	Approximate LOD (cfu/test)
B. anthracis (crude, not heat shocked)	1.5 x 10E8 1.5 x 10E7 1.5 x 10E6 1.5 x 10E5 7.5 x 10E4 3.8 x 10E4 1.9 x 10E4 1.5 x 10E4 1.5 x 10E3	1/1 1/1 1/1 1/1 3/3 0/1 0/1 0/1 0/1	7.5 x 10E4	7.5 x 10E2
B. anthracis (washed, heat shocked)	2.5 x 10E7 2.5 x 10E6 1.25 x 10E6 6.3 x 10E5 3.1 x 10E5 1.6 x 10E5 8.0 x 10E4 2.5 x 10E5 2.5 x 10E4	1/1 1/1 1/1 1/1 1/1 2/3 0/1 0/1 0/1	3.1 x 10E5	3.1 x 10E3

Results obtained by RAMP™ analysis of closely related live *Bacillu*s strains, *B. globigii*, *B. cereus*, and *B. thuriengiensis*, are presented in Table 3. The RAMP™ anthrax assay did not react with live *B. globigii* and *B. thuringiensis* at concentrations up to 2.0 x 10E9 cfu/mL (2.0 x 10E7 cfu/test) and 1.5 x 10E9 cfu/mL (1.5 x 10E7 cfu/test), respectively. At 2 x 10E10 cfu/mL (2 x 10E8 cfu/test), *B. globigii* cross-reacted in the *B. anthracis* assay. This result is not unexpected, as *Bacillus* strains, particularly *B. globigii*, *B. cereus*, and *B. thuringiensis* differ only slightly at the gene level from *B. anthracis* (15, 16, 17). *B. cereus* was negative by RAMP™ assay at 1.1 x 10E4 cfu/mL (1.1 x 10E2 cfu/test); this is probably because the concentration of *B. cereus* at this concentration was below the LOD of the RAMP™ assay. Normally, one would expect to propagate a *B. cereus* culture for about two weeks to acquire a high concentration of this organism. However, due to time constraints on the present study, *B. cereus* was grown for only 6 days.

Table 3. RAMP™ analysis of closely related live Bacillus strains

Agent	Concentration (cfu/mL)	Positive/Total
B. globigii	2 x 10E10 2 x 10E9	6/6 0/4
B. cereus	1.1 x 10E4	0/3
B. thuringiensis	1.5 x 10E10 1.5 x 10E9 1.5 x 10E8	-/3* 0/3 0/3

* too concentrated; unable to read

Of six negative (no antigen) controls, five were negative and one was positive (false positive) (data not shown). It was later learned that the sample that had produced a false positive result had been diluted in water, rather than BioVeris sample buffer. Whether this may have caused the false positive result is unknown. The fluorimeter functioned well with one exception, when the instrument would not accept several cartridges for reading. This problem was solved by turning the reader off and on and re-reading the cartridge, with the exception of one cartridge which could not be read and may have been faulty. Also, on one occasion, a cartridge could not be read because the sample was deemed to be too concentrated. On consultation with Response Biomedical Corp., we were informed that under normal end-user operational procedures (when samples are timed internal to the reader), the reader would flag a sample that is too viscous or concentrated and would request that it be diluted.

The LODs of the RAMP™ assay for *B. anthracis* observed in this study are similar to those reported by Response Biomedical from their in-house studies, where an LOD of 4.7 x 10E3 cfu/test was reported for *B. anthracis* (Sterne vaccine) (live, attenuated), and no cross-reactivity with *B. thuringiensis* or *B. globigii* at concentrations up to 1 x 10E8 cfu/test sample (18). Similarly, in independent studies conducted by a Maryland state laboratory, the LOD of RAMP™ for detection of *B. anthracis* (live) was found to be 4.0 x 10E3 cfu/test (B. Radvak, Response Biomedical Corp., personal communication). These reported LODs would suggest that the RAMP™ anthrax assays were at least 100 times more sensitive in detection of *B. anthracis* spores than were competitive commercial chromogenic HHAs, where LODs of 10E5-10E8 spores/test have been reported (19, 20). The infective dose of *B. anthracis* is not precisely known, however, the ID50 for inhalation anthrax is generally regarded to be in the range of 8,000 to 50,000 spores (21), while the LD_{50} for primates has been reported at between 2,500 and 55,000 spores (22, 23). Thus, the sensitivity of the RAMP™ assay for anthrax appears to be in the range of that required to detect the estimated ID_{50} or LD_{50} of live *B. anthracis*. Furthermore, this study suggests that the RAMP™ system offers enhanced sensitivity over conventional chromogenic HHAs for identification of *B. anthracis*.

Based on the results of this present study and subsequent studies that have been conducted to evaluate the RAMP™ system, the RAMP™ is suggested as a valuable alternative to chromogenic HHAs for rapid, on-site detection of *B. anthracis* by First Responers and military personnel. At the time of writing, the RAMP™ system has been adopted for use by the Royal Canadian Mounted Police and First Responders for several Canadian cities and police departments (B. Radvac, personal communication).

4 Summary

The RAMP™ system was evaluated for sensitivity and specificity in detection of *B. anthracis* by challenging with killed and live *B. anthracis* and closely related *Bacillus* strains in the BSL2 and BSL3 laboratories at DRDC Suffield. The LOD of RAMP™ HHAs for detection of killed *B. anthracis* was 2.6 x 10E6 cfu/mL, corresponding to 2.6 x 10E4 cfu/test. Live *B. anthracis* (washed, heat shocked) and crude *B. anthracis* (not washed, not heat shocked) were detected at 3.1 x 10E5 cfu/mL and 7.5 x 10E4 cfu/mL, respectively; these values correspond to a LOD per test of 3.1 x 10E3 cfu and 7.5 x 10E2 cfu, respectively. The RAMP™ system did not cross-react with other closely related *Bacillus* strains at the concentrations examined, with the exception of *B. globigii*, which cross-reacted at a concentration in excess of 2.0 x 10E9 cfu/mL. The RAMP™ system has been shown to be a valuable alternative to chromogenic HHAs for rapid, on-site detection of *B. anthracis* by First Responders and military personnel.

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Figure 1. Diagrammatic representation of the operational procedures used in the RAMP™ system (reproduced courtesy of W. Radvak, Response Biomedical Corp.).

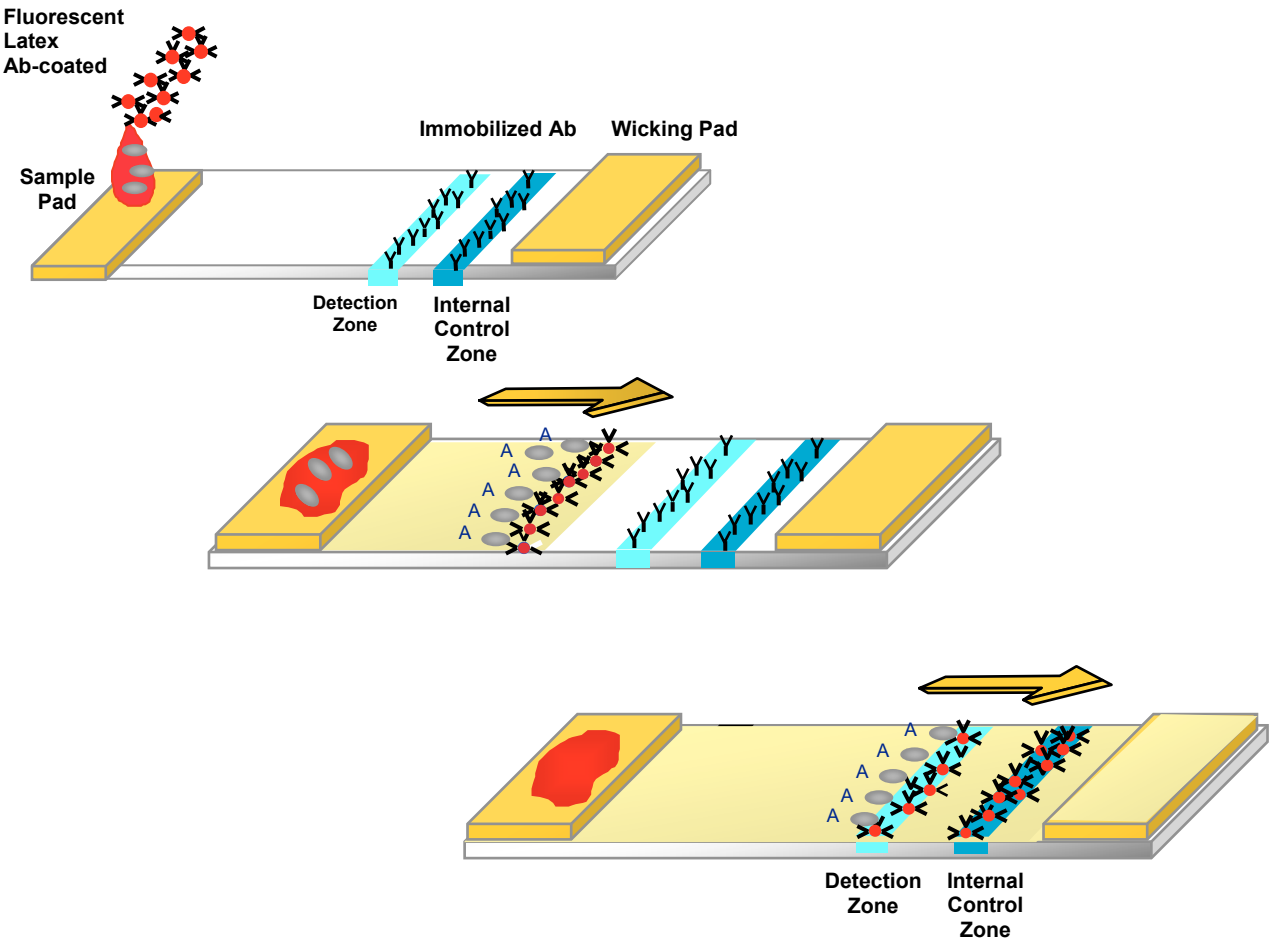

Figure 2. Diagrammatic representation of the principle of operation of the RAMP™ system (reproduced courtesy of W. Radvak, Response Biomedical Corp.)

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The Rapid Analyte Measurement Platform (RAMP™) hand-held assay (HHA) system was evaluated for sensitivity and specificity in detection of *B. anthracis* by challenging with killed and live *B. anthracis* and closely related *Bacillus* strains in the BSL2 and BSL3 laboratories at DRDC Suffield. The LOD of RAMP™ HHAs for detection of killed *B. anthracis* was 2.6 x 10E6 cfu/mL, corresponding to 2.6 x 10E4 cfu per test. Live *B. anthracis* (washed, heat shocked) and crude *B. anthracis* (not washed or heat shocked) were detected at 3.1 x 10E5 cfu/mL and 7.5 x 10E4 cfu/mL, respectively; these values correspond to a LOD per test of 3.1 x 10E3 cfu and 7.5 x 10E2 cfu, respectively. The RAMP™ system did not cross-react with other closely related *Bacillus* strains at the concentrations examined, with the exception of *B. globigii*, which cross-reacted at a concentration in excess of 2.0 x 10E9 cfu/mL. The RAMP™ system may be a valuable alternative to chromogenic HHAs for rapid, on-site detection of *B. anthracis* by First Responders and military personnel.

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