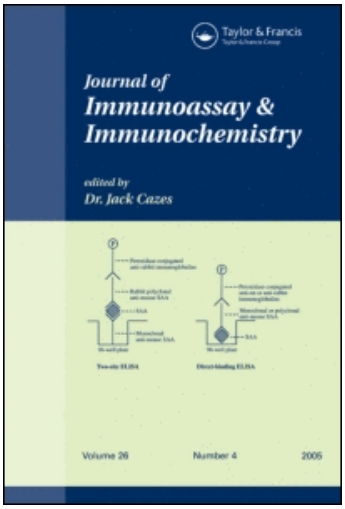

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Fluorogenic Hand-Held Immunoassay for the Identification of Ricin: Rapid Analyte Measurement Platform

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Fluorogenic Hand-Held Immunoassay for the Identification of Ricin: Rapid Analyte Measurement Platform

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Abstract: Rapid analyte measurement platform (RAMP^{TM}) fluorogenic hand-held immunoassays (HHAs) were evaluated for inclusivity/sensitivity, exclusivity/specificity, sample matrix effects, ruggedness/stability, and reproducibility in detection of ricin (RCA_{60}), a potential biological threat agent. The limit of detection of HHAs for RCA_{60} was 14 ng/mL or approximately 140 pg/test. HHAs were inclusive in detection of ricin RCA_{60}, RCA_{120}, ricin A chain, and ricin B chain and exclusive in discrimination of RCA_{60} from other toxins. None of the sample matrices tested affected assay performance. RAMP^{TM} ratios were tolerant of increases in sample volume, however, a decrease in sample volume of 25% resulted in significantly increased readings. RAMP^{TM} readings were highly reproducible lot-to-lot, cartridge-to-cartridge, day-to-day, and reader-to-reader.

Keywords: Hand-held assay, Ricin, Rapid analyte measurement platform, RAMP^{TM}, Immunochromatographic assay, *Ricinus communis*, Biological threat agent

INTRODUCTION

Hand-held immunoassays (HHAs) are rapid-response, disposable devices similar in basic technology to home pregnancy test kits. The technique is based on a lateral flow, immunochromatographic procedure that utilizes the binding properties of specific antibodies with their corresponding antigens. The advantages of HHAs over more traditional immunoassays include ease

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of operation, short reaction time, and relative low cost per unit assay, enabling economic on-site testing. HHAs are viewed as ideal to meet the needs of military personnel and First Responders for front-line detection and identification of biological threat agents. Preliminary identification of biological agents with these devices would allow for advance initiation of appropriate medical countermeasures and would typically be followed by confirmatory identification using other, laboratory-based technologies. HHAs for the identification of a number of potential biological threat agents, including *Bacillus anthracis*,[1–4] ricin,[5] *Francisella tularensis*,[6] *Burkholderia mallei*,[7] Staphylococcal enterotoxin B,[8] *Brucella spp.*,[9] *Yersinia pestis*,[2] and aflatoxin,[10] have been described. A review of immunochromatographic assays detection and identification of infectious diseases and biological warfare agents has also been published.[11]

Our institution has evaluated a number of HHA technologies based on lateral flow chemistries for detection and identification of biological agents. One such technology is the Rapid Analyte Measurement Platform (RAMP^{TM}), a fluorescence-based identification system developed by Response Biomedical Corp. (Burnaby, BC). A fluorescence approach to detection of analyte is theoretically more sensitive than chromogenic approaches that are utilized in more conventional HHA systems. Preliminary studies at our institution on evaluation of RAMP^{TM} for identification of *Bacillus anthracis* have confirmed this finding.[4] The purpose of the present study was to comprehensively evaluate the RAMP^{TM} system for the identification of another potential biological threat agent, ricin. Ricin has long been considered a potential agent of biological warfare and bioterrorism.[12,13] Furthermore, ricin is of current concern as a potential biological threat agent as it has been detected in envelopes handled by the US postal system.[14]

EXPERIMENTAL

RAMP^{TM} System

The RAMP^{TM} 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.

Two RAMP™ readers, RAMP™ data collection software (V210.exe), RAMP™ ricin cartridges, and RAMP™ ricin lot cards were purchased from Response Biomedical Corp.

Antigens and Antibodies

Working stock concentrations of antigens were prepared in advance by dilution of original stocks in phosphate buffered saline, pH 7.4 (PBS) and stored at 4°C until used. RCA_{60}, RCA_{120}, ricin A chain, and ricin B chain were purchased from Sigma-Aldrich Canada (Oakville, ON). Prior to use, RCA_{60}, RCA_{120}, ricin A chain, and ricin B chain were diluted to working stock concentrations of 39, 55.6, 71, and 43 μg/mL, respectively. Botulinum toxins A-F, respectively, were purchased from Wako Chemicals Inc. (Richmond, VA) and were pooled and diluted to a working stock concentration of 1 μg/mL. Staphylococcus enterotoxin B (SEB) was purchased from Toxin Technologies (Sarasota, FL) and was diluted to a working stock of 10 μg/mL. For the RAMP™ assays, dilutions of antigen working stocks were made in PBS containing 0.5% BSA (PBS-BSA) and, unless otherwise noted, were prepared fresh on the day on which they were tested.

Antibodies incorporated in RAMP™ cartridges were: detector and capture anti-ricin antibodies, both produced in rabbit, and control antibody, anti-rabbit IgG. The source of these antibodies was unknown.

Buffers and Reagents

PBS was purchased from Sigma-Aldrich Canada. Bovine serum albumin was purchased from Roche Diagnostics Canada (Laval, QC). RAMP™ buffer was purchased from Response Biomedical Corp. Flour (white, enriched, all purpose, Safeway brand), cornstarch (Safeway brand), baking powder (Safeway brand), laundry detergent (Tide, Original, Procter and Gamble), and coffee creamer (Coffee-Mate®, Carnation, Nestlé) were purchased from Safeway Canada Ltd. (Medicine Hat, AB).

General Procedures

Protocols developed for evaluation of RAMP™ for identification of ricin followed as closely as possible those endorsed by the Association of Analytical Communities (AOAC) International[15] for validation of rapid, immunoassay-based *Bacillus anthracis* detection systems (P. Emanuel, personal communication).

Two RAMP™ readers were placed within a Class II Biosafety Cabinet; each reader was connected to a computer located outside the Cabinet. Preliminary experiments conducted prior to commencement of this study indicated no significant difference between RAMP™ ratios obtained on the two readers used in the study (standard deviation of means test). Both readers were configured for automatic timing of assay reactions within the reader. Unless otherwise stated, samples were assayed in replicates of five cartridges, simultaneously and consecutively, on the two readers, in a single day.

Procedures for performance of RAMP™ assays were as suggested by Response Biomedical Corp. and were as follows. A 10 μL aliquot of sample was transferred to a microfuge tube containing 90 μL of RAMP™ sample buffer. The RAMP™ cartridge and accompanying pipette tip were removed from the cartridge pouch. The pipette tip was fitted to a variable volume Gilson Pipetman pipette (Mandel Scientific, Guelph, ON) and the sample was mixed thoroughly by triturating 15 times. A 70 μL volume of the sample was delivered to the sample well and the cartridge was inserted into the RAMP™ reader. After the reader had detected sample flow ("sample flow detected" message), the assay was allowed to develop within the reader for 12.5 min. The reader then scanned the cartridge and the raw data was automatically sent to the computer for reduction (conversion to RAMP™ ratio) and analysis.

Inclusivity/Sensitivity

Experiments were designed to determine the limit of detection (LOD) of RAMP™ ricin HHAs for a variety of types and components of ricin. Varying concentrations of RCA_{60} (5–1000 ng/mL), RCA_{120} (5–1000 ng/mL), ricin A chain (5–1000 ng/mL), and ricin B chain (25–2000 ng/mL) were prepared in sterile PBS-BSA. Antigen solutions were prepared one day prior to use and stored overnight at 4°C. Assays were conducted over the course of four consecutive days.

Exclusivity/Specificity

Experiments were designed to assess the specificity of RAMP™ ricin HHAs in discriminating ricin from other potential biothreat toxins (SEB and botulin)

and in detecting ricin in the presence of these other toxins. Working solutions of botulin (A-F combined) and SEB, respectively, were prepared in PBS-BSA at concentrations of 1x, 10x, and 70x LOD RCA_{60}, as determined from the inclusivity/sensitivity data, above. To spike botulin and SEB with ricin, sufficient RCA_{60} to obtain final concentrations of 10x LOD RCA_{60} was added to aliquots of each 70x SEB and botulin, respectively. The negative control was sterile PBS-BSA. The positive control was RCA_{60} at 10x LOD.

Matrix Effects

Experiments were designed to evaluate the performance of RAMP™ ricin HHAs in the presence of a variety of matrix materials. RAMP™ HHAs were tested with the following powders, both alone and in the presence of RCA_{60}: flour, cornstarch, baking powder, laundry detergent, and coffee creamer. From information provided by the manufacturer, approximately 2 mg of powder is transferred to 150 μL of RAMP™ buffer when the sampling swab supplied with the commercial RAMP™ kit is used (Response Biomedical Corp., personal communication). Thus, powder samples were prepared in bulk in RAMP™ buffer to equal final concentrations of 2 mg powder per 150 μL buffer. Sufficient RCA_{60} was added to half of the bulk powder mixtures to equal 10x LOD RCA_{60}. An aliquot of 70 μL of the powder mixture (with or without RCA_{60}) was added to the test cartridge.

Ruggedness/Stability

Experiments were designed to evaluate the effect of variability in sample volume, by determining RAMP™ ratios when sample volume was increased by 25% or decreased by 25%, and by comparing with RAMP™ ratios when the standard sample volume of 70 μL was used. RCA_{60} (10x LOD) and negative controls (PBS-BSA) were tested on cartridges of the same manufacturing lot. A 10 μL sample aliquot was added to 90 μL RAMP™ buffer, mixed, and a volume of 70 μL, 87.5 μL, or 52.5 μL was added to the cartridge.

Reproducibility and Accuracy

Lot-to-lot reproducibility was assessed by testing two different manufacturing lots of RAMP™ cartridges with 10x LOD RCA_{60} in replicates of 12, six on each of the two readers, and with negative controls (PBS/BSA) in replicates of six, three on each of the two readers. Cartridge-to-cartridge reproducibility was assessed by analysis of the data from single manufacturing lots, in the

lot-to-lot experiments described above. Coefficients of variation (CVs) were determined for replicates of 12 assays of 10x LOD RCA_{60} on each of the two lots of cartridges. Day-to-day reproducibility was determined by testing 10x LOD RCA_{60} and negative controls (PBS-BSA) in replicates of five, two on one reader and three on the other reader, on cartridges of the same manufacturing lot on five different days. Reader-to-reader reproducibility was assessed by examination of RAMP™ ratios obtained on each of the two readers over the course of this study, on all cartridges from the same manufacturing lot in which 10x LOD RCA_{60} and PSB/BSA controls were tested.

Analysis of Data and Statistics

Data from the RAMP™ readers was converted via the RAMP™ software to RAMP™ ratio. The RAMP™ ratio is defined as: (TL-MB) divided by [(TL-MB) + (ISL-MB)] × 60,000, where TL = signal at test line, MB = mean background signal, ISL = signal at internal standard line, and 60,000 is a constant. RAMP™ ratios were exported to Microsoft Excel 7.0 for reduction and Grapher 4 (Golden Software Inc., Golden, CO) for plotting of graphs and curve fit analysis. Each set of data points was analyzed by Chauvenet's criterion[16] to eliminate outlying data points. Assay cut-off was defined as the average negative control signal plus two standard deviations (sd) (0 ng/mL + 2sd) (high stringency) or, twice the average negative control signal (2 × 0 ng/mL) (low stringency), the latter as recommended by the manufacturer for use in the field (Response Biomedical Corp., personal communication). LODs were determined graphically by plotting the best fit curve and recording the intersection of the curve with the assay cut-off. The difference between RAMP™ ratio means was assessed for significance by usage of a standard deviation of the means test, wherein it can be estimated that two means are significantly different if the difference between them is greater than twice the sum of their standard deviations.[16]

RESULTS

Inclusivity/Sensitivity

RAMP™ ratios were plotted as a function of concentration of RCA_{60} (Figure 1). LODs were determined graphically from the intersection of the best fit curve (in all cases quadratic) with the assay cut-off. The LODs for RCA_{120}, ricin A chain, and ricin B chain were similarly determined. LODs obtained in this manner are summarized in Table 1.

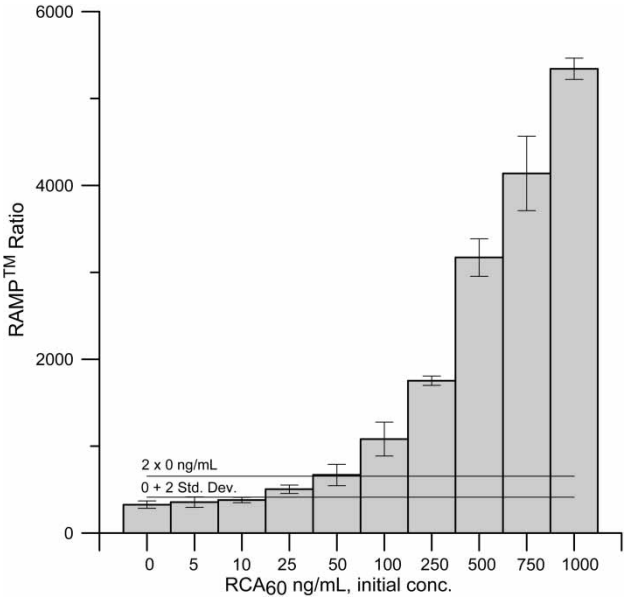

Figure 1. Inclusivity/sensitivity: LOD of RAMP^{TM} assay for detection and identification of RCA_{60} at two levels of stringency (high stringency: 0 ng/mL + 2 sd; low stringency: 2 × 0 ng/mL). Error bars represent the mean +/− one standard deviation.

Exclusivity/Specificity

RAMP^{TM} ratios were plotted as a function of concentration of botulin A-F and SEB, respectively, both in the absence and presence of RCA_{60} (Figure 2). Neither botulin nor SEB produced signals above the negative control at either level of stringency. In addition, RCA_{60} in the presence of botulin or SEB was detected at the same RAMP^{TM} ratio as that of RCA_{60} alone (sd of means test).

Table 1. LODs (ng/mL) for RAMP^{TM} ricin assays at two levels of stringency

RCA_{60}	RCA_{120}	Ricin A chain	Ricin B chain
Cut-off = 0 ng/mL + 2sd			
14	16	1	350
Cut-off = 2 × 0 ng/mL			
47	135	33	870

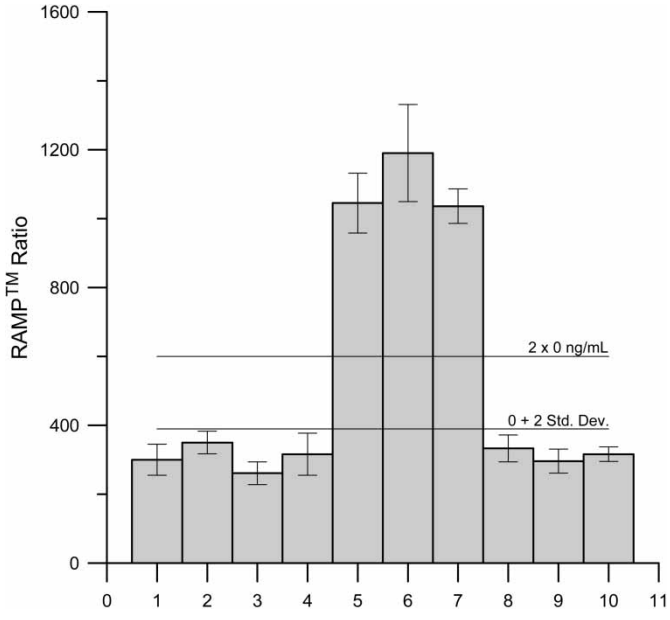

Figure 2. Exclusivity/specificity: Specificity of RAMP™ assay in discrimination of RCA_{60} from other toxins (botulin and SEB). 1: PBS-BSA; 2–4: botulin at 1x, 10x, and 70x LOD RCA_{60}, respectively; 5: botulin at 70x LOD RCA_{60} + 10x LOD RCA_{60}; 6: 10x LOD RCA_{60} (positive control); 7: SEB at 70x LOD RCA_{60} + 10x RCA_{60}; 8–10: SEB at 1x, 10x, and 70x LOD $RCA_{60,}$ respectively. 1x, 10x, and 70x LOD RCA_{60} = 14, 140, and 980 ng/mL, respectively. Error bars represent the mean +/− one standard deviation.

Matrix Effects

RAMP™ ratios obtained with samples containing flour, cornstarch, baking powder, laundry detergent, and coffee creamer, both in the absence and presence of ricin RCA_{60}, are presented in Figure 3. None of the powders in the absence of RCA_{60} produced RAMP™ ratios that were above the assay cut-offs at either level of stringency and none of the signals was significantly different than the PBS-BSA control (sd of means test). In addition, each of the respective powders spiked with RCA_{60} produced RAMP™ ratio signals that were not significantly different from that produced by RCA_{60} alone (positive control) (sd of means test).

Ruggedness/Stability

Results of experiments to determine the effect of variations in sample volume on RAMP™ ratios, when sample volume was increased or decreased by 25%

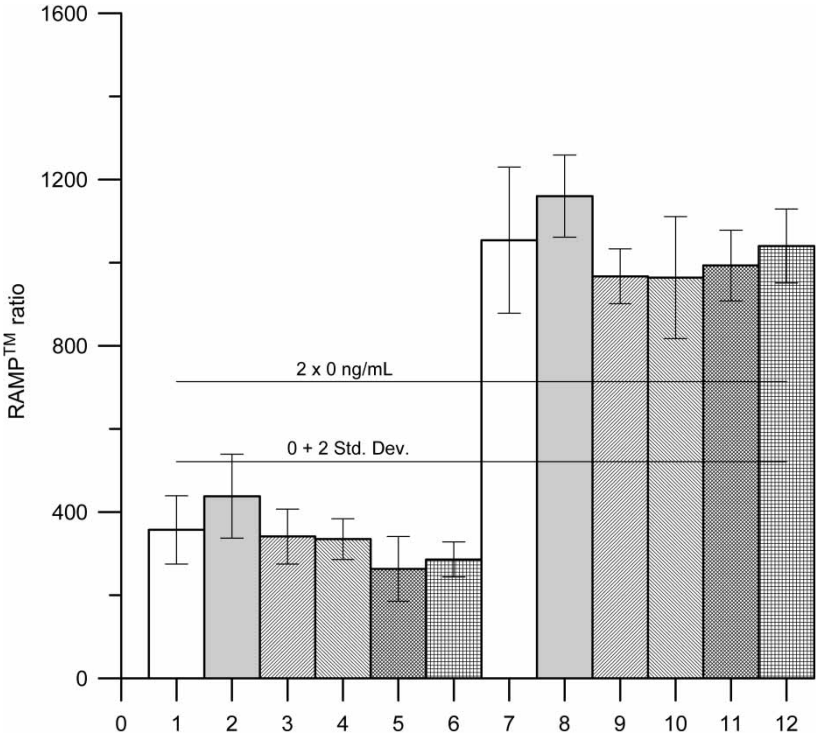

Figure 3. Matrix effects: RAMP^{TM} assay of RCA_{60} in presence and absence of various powder matrices. 1–6: powder matrices only; 7–12: powder matrices spiked with 10x LOD RCA_{60} (140 ng/mL); ☐: buffer; ▒: flour; ▨: corn starch; ▧: baking powder; ▩: laundry detergent; ▦: creamer. Error bars represent the mean +/− one standard deviation.

from the standard volume, are presented in Figure 4. No difference in RAMP^{TM} ratio was found between assays that received the standard 70 μL sample volume and those that received 87.5 μL (+25%) of either buffer or RCA_{60} (sd of means test). However, a significant increase in RAMP^{TM} ratio was observed for both the buffer and RCA_{60} samples when the sample volumes were decreased to 52.5 μL (−25%) (sd of means test).

Reproducibility and Accuracy

Lot-to-lot reproducibility was assessed by comparing RAMP^{TM} ratios obtained on cartridges from two different manufacturing lots. Results indicated no significant difference in RAMP^{TM} ratio between the two different manufacturing lots tested (sd of means test). CVs between lots for 10x LOD RCA_{60} and buffer controls were 13.2% and 17.5%, respectively.

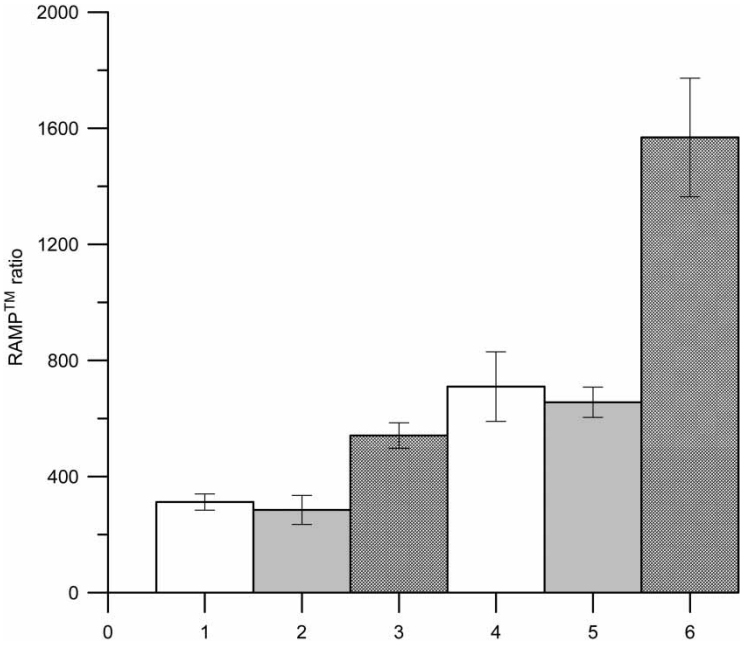

Figure 4. Ruggedness/stability: Effect of variations in sample volume on performance of RAMP™ assay in detection and identification of RCA_{60}. 1–3: PBS-BSA; 4–6: 10x LOD RCA_{60} (140 ng/mL); ▭: 70 μL (standard); ▭: 87.5 μL (+25%); ▭: 52.5 μL (−25%). Error bars represent the mean +/ − one standard deviation.

Cartridge-to-cartridge reproducibility was assessed by comparing RAMP™ ratios obtained on replicate assays on cartridges from each of the two manufacturing lots. Observed CVs in RAMP™ ratios for 10x LOD RCA_{60} on lot 1 cartridges were 8.4% and 18%, respectively; on lot 2 cartridges CVs were 18% and 17%, respectively. Day-to-day reproducibility was assessed by comparing RAMP™ ratios obtained on replicate assays performed on different days over the course of several months (Figure 5a). By the sd of means test, no significant difference in RAMP™ ratios was found between assays performed on the days examined. CVs in RAMP™ ratios among days for 10x LOD RCA_{60} and buffer controls were 12.5% and 14.2%, respectively. Reader-to-reader reproducibility was assessed by comparing RAMP™ ratios obtained on replicate assays performed using the two different RAMP™ readers employed in this study (Figure 5b). No difference was found between assays performed on the two readers (sd of means test). CVs in RAMP™ ratios between readers for 10x LOD RCA_{60} and buffer controls were 12.5% and 14.2%, respectively.

Chauvenet's criterion was used to exclude outlying data points, resulting in an overall (inclusive of all experiments) rejection rate of 4.8%. Overall CVs

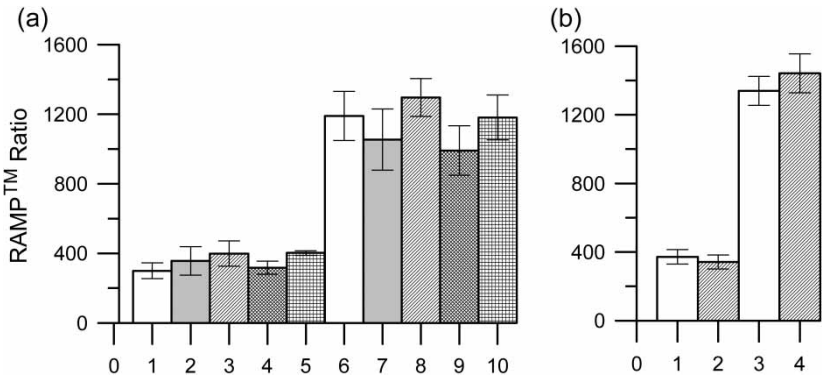

Figure 5. Reproducibility: RAMP™ assay of RCA_{60} in cartridges read on (a) different days. 1–5: PBS-BSA; 6–10: 10x RCA_{60} (140 ng/mL); ▭: 05.01.25; ▭: 05.01.31; ▭: 05.02.15; ▭: 05.06.15; ▭: 05.06.16. (b) different readers. 1–2: PBS-BSA; 3–4: 10x RCA_{60} (140 ng/mL); ▭: reader 1; ▭: reader 2. Error bars represent the mean $+/-$ one standard deviation.

among replicates ranged from 0.7 to 31% with both the median and mean CV being 11%.

DISCUSSION

Ricin, also referred to as *Ricinus communis* Agglutinin II (RCA II) or RCA_{60}, is a toxic glycoprotein lectin derived from the seeds of *Ricinus communis* or castor bean plant. Ricin consists of two disulfide-linked polypeptide chains, termed A chain and B chain, of ~28 kDa and 32 kDa, respectively.[17] The B chain binds to cell surfaces via galactose residues and facilitates transport of the lectin into the cell. The A chain has enzymatic activity which inhibits protein synthesis by depurination of adenine residues in 28s ribosomes.[18] The A chain, when separated from the B chain, is incapable of entering the cell and is thus non-toxic. RCA_{120}, also referred to as *Ricinus communis* Agglutinin I (RCA I), is a moderately toxic glycoprotein also sourced from *Ricinus communis* castor bean seeds. It has a molecular weight of 120 kDa and consists of four subunits, two A chains and two B chains. RCA_{120} is slightly less toxic than RCA_{60} and causes agglutination of mammalian red blood cells.[19]

The events of 11 September 2001 and subsequent incidents involving anthrax-laced letters in the U. S. postal system were followed by a proliferation of commercial firms marketing HHAs for rapid identification of anthrax, in an industrial environment that was largely unregulated. As a result, the need for regulation of biodetection devices and procedures was recognized and led to the development of performance standards endorsed

by the AOAC International[15] for validation of rapid, immunoassay-based *Bacillus anthracis* detection systems.[20] The RAMP™ system used in this present study underwent AOAC test and evaluation and was the only hand-held device to meet AOAC performance standards for detection of anthrax.[21] For the present study, the AOAC International performance standards for anthrax detection systems were followed as a guideline in development of protocols for validation of the RAMP™ HHAs for ricin. For the ricin study, modifications to the anthrax protocols were necessitated by the nature of the analyte and the scope of the study.

Evaluation of the inclusivity/sensitivity of RAMP™ ricin HHAs cartridges was undertaken to establish the minimum amount of RCA_{60}, RCA_{120}, A chain, and B chain required to elicit a positive assay result. Two levels of stringency were used in determining the LODs of RAMP™ ricin assays, a high stringency cut-off (0 ng/mL+2 sd) and a low stringency cut-off (2×0 ng/mL). The low stringency cut-off was recommended by the manufacturer for use by First Responders in the field. However, in the present study, RAMP™ ratios observed for sample matrix and heterologous toxin controls were always well below the high stringency cut-off. At the high stringency level, the assay detected RCA_{60}, RCA_{120}, ricin A chain, and ricin B chain at 14, 16, 1, and 350 ng/mL, corresponding to approximately 0.14, 0.16, 0.01, and 3.5 ng/test, respectively. A fluorescence-based approach such as that provided in the RAMP™ HHA system was expected to be more sensitive than the chromogenic approach that is used more conventionally in HHA systems. This expectation was confirmed in the present study with the finding that the sensitivity of the RAMP™ HHA system for RCA_{60} was 14 ng/mL. This LOD may be compared with a LOD of 50 ng/mL reported by Shyu et al. for a colloidal gold-based HHA for ricin,[5] the only reported HHA for ricin previously described in the literature. The LD_{50} (mouse) of RCA_{60} is estimated at 3 μg/kg (ingestion, inhalation, and injection).[22] By linear extrapolation and assuming an average human weight of 68 kg, the LD_{50} (human) may be estimated at ~204 μg. Regardless of assay stringency (low or high), the RAMP™ assay for ricin could detect RCA_{60}, RCA_{120}, ricin A chain, and ricin B chain at concentrations well below the human toxic dose of RCA_{60}, the most toxic of the ricin forms.

The effect of potential interfering substances on RAMP™ assay performance was assessed by testing solutions of various powders, both in the presence and absence of ricin. None of the powders in the absence of ricin produced false positive signals. Similarly, there was no effect of any of the powders on the signal for ricin, i.e., no depression or increase in RAMP™ ratio compared to the ricin positive control. This observation suggests that RAMP™ ricin assays are tolerant of samples containing matrix materials such as powders. To confirm this finding, evaluation of additional types of powder matrices is warranted. In addition, because many potential samples contain soil, it would be valuable to evaluate RAMP™ assays for tolerance to soil matrices.

In studies to determine the effect of variations in sample volume added to the cartridge, it was found that there was no effect on RAMP™ ratio when the sample volume was increased by 25%, but when sample volume was decreased by 25%, a significant increase in RAMP™ ratio occurred. This observation may be analyzed by examining the mechanism used to calculate RAMP™ ratio. The RAMP™ ratio is essentially the signal from the test line (TL) divided by the sum of the signals from the test line and internal standard line (ISL). Whether buffer or ricin was applied to the cartridge, the ISL signal was typically observed to be unchanged when the volume of the sample did not change (data not shown). When increased sample volume of either buffer or ricin was applied to the cartridges, the signals for both the TL and ISL either increased or remained unchanged (data not shown), thus resulting in a RAMP™ ratio that was unaffected. However, when the sample size of buffer or ricin was too small, the ISL was lower than that obtained when the standard 70 μL was added, while the TL remained unchanged (data not shown). This may have occurred as a result of binding being complete at the TL but incomplete at the ISL, located further down the wicking path. Thus, the ISL would not be fully developed, resulting in a lower ISL signal and an increased RAMP™ ratio. The finding that reduction in sample volume by 25% from the standard sample volume resulted in an increase in RAMP™ ratio suggests that an operator error of this magnitude could possibly lead to conclusion of a false positive result. Although operator error of this magnitude is unlikely, it would be useful to know how much of a measurement error could be tolerated before this would be manifested in an erroneous reading.

RAMP™ ratios were highly reproducible lot-to-lot, cartridge-to-cartridge, day-to-day, and reader-to-reader. There was no significant variability (100% repeatability) in RAMP™ ratios regardless of whether assays were performed on the same day, on different days, on different lot numbers, or on different readers.

CONCLUSIONS

RAMP™ HHAs were evaluated for inclusivity/specificity, exclusivity/specificity, sample matrix effects, ruggedness/stability, and reproducibility in detection and identification of ricin. RAMP™ HHAs were inclusive in detection of RCA_{60}, RCA_{120}, ricin A chain, and ricin B chain at LODs of 14, 16, 1, and 350 ng/mL, respectively. RAMP™ HHAs detected ricin exclusive of other toxins; there was no cross-reactivity with other toxins tested and assay LOD for RCA_{60} was unaffected in the presence of other toxins. None of the sample matrices tested had an effect on assay performance. RAMP™ ratios were tolerant to increases in sample volume, although a decrease in sample volume of 25% resulted in significantly increased readings. RAMP™ readings were highly reproducible lot-to-lot, cartridge-

to-cartridge, day-to-day, and reader-to-reader. Results of the present study suggest that the RAMP™ fluorogenic HHA system may be a valuable alternative to chromogenic HHAs for use by military personnel and First Responders for in-field detection and identification of ricin.

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