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Development of Sampling Methods for Powders and Soil for Detection of Biothreat Agents by Electrochemiluminescence

H.G. Thompson, R.E. Fulton, and J. Ratches
DRDC Suffield

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DRDC Suffield TM 2007-172
June 2007

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Abstract

The M1M (BioVeris Corporation), an automated electrochemiluminescent (ECL) assay instrument, has been shown to be a technology that is robust and tolerant of samples in a variety of complex matrices; however, methods for sampling of powders and soils in the field for processing on the M1M have not been described. This report describes the development of simple and reproducible methods that may be used for field sampling of powders and soils for subsequent screening and analysis on the M1M. By sampling with polyester swabs using a standard method, it was found that it was possible to collect a reproducible amount (~12 mg) of powder or soil from a variety of surfaces. Using this value, the volume of diluent required to produce a signal <1.2 on the M1M was empirically determined. The powder or soil suspension was filtered and the filtrate assayed on the M1M. The result was a method that could be used consistently to assay powder and soil by ECL M1M with good sensitivity and minimal background signal.

Résumé

Le M1M (BioVeris Corporation), un instrument automatisé de bioessais électrochimiluminescents (ECL) s'avère être une technique robuste et tolérante des échantillons, dans une variété de matrices complexes; on n'avait cependant pas encore décrit les méthodes d'échantillonnage sur le terrain des poudres et des sols devant être traités sur le M1M. Ce rapport décrit la mise au point de méthodes simples et reproductibles pouvant être utilisées pour l'échantillonnage sur le terrain des poudres et sols, ultérieurement criblés et analysés sur le M1M. On a trouvé qu'en utilisant la méthode standard d'échantillonnage avec des porte-coton en polyester, il était possible de recueillir une quantité reproductible (~12 mg) de poudre ou sol provenant d'une variété de surfaces. On a déterminé empiriquement, en utilisant cette valeur, le volume de diluant requis pour produire un signal <1,2 sur le M1M. La poudre ou sol en suspension a été filtré et le filtrat bio-testé sur M1M. On en conclut que la méthode peut être utilisée régulièrement pour bio-tester les poudres et sols sur le M1M ECL avec une bonne sensibilité et un signal de fond minimal.

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Executive summary

Development of Sampling Methods for Powders and Soil for Detection of Biothreat Agents by Electrochemiluminescence

H.G. Thompson, R.E. Fulton, and J. Ratches; DRDC Suffield TM 2007-172;
Defence R&D Canada – Suffield; June 2007.

Introduction or background: The M1M (BioVeris Corporation), an automated electrochemiluminescent (ECL) assay instrument, is currently being considered by several agencies for field identification of biothreat agents. The technology employed has been shown to be robust and tolerant of samples in a variety of complex matrices. However, methods for sampling of powders and soils in the field for processing on the M1M had not been developed previously.

Results: A method for collecting reproducible amounts of powder or soil from a variety of surfaces using polyester swabs was devised. Relatively constant sample size permitted a simple, standard method for sample preparation to be developed. The overall protocol allows suspect powders and soil to be assayed by the M1M with good sensitivity and minimal background signal.

Significance: The sampling protocol developed here, and the operation of the M1M, are rapid, relatively simple, and easily transferable from a laboratory setting to the field. Their adoption by the Canadian Forces and by first responders would afford a markedly improved ability to detect and identify agents of biological origin.

Future plans: The study will be expanded to include other powders and soil types, as well as other biothreat agents.

Sommaire

Development of Sampling Methods for Powders and Soil for Detection of Biothreat Agents by Electrochemiluminescence

H.G. Thompson, R.E. Fulton, and J. Ranches; DRDC Suffield TM 2007-172; R & D pour la défense Canada – Suffield; juin 2007.

Introduction ou contexte: Le M1M (BioVeris Corporation) un instrument automatisé de bioessais électrochimiluminescents (ECL), est actuellement considéré par plusieurs agences pour l'identification, sur le terrain, d'agents représentant une menace biologique. La technologie employée s'avère robuste et tolérante des échantillons dans une variété de matrices complexes. On n'avait cependant pas décrit auparavant les méthodes d'échantillonnage sur le terrain des poudres et des sols devant être traités sur le M1M.

Résultats: On a conçu une méthode, visant à recueillir les quantités reproductibles de poudre ou sols provenant d'une variété de surfaces, au moyen de porte-coton en polyester. La taille relativement constante des échantillons a permis de mettre au point une méthode standard de préparation d'échantillons. Le protocole général permet de bio-tester les poudres et sols suspects sur le M1M avec une bonne sensibilité et un signal de fond minimal.

Portée des résultats: Le protocole d'échantillonnage mis au point ici ainsi que l'opération du M1M sont rapides et relativement simples et facilement transférables d'un contexte de laboratoire à celui du terrain. L'adoption de ce protocole par les Forces canadiennes et les premiers intervenants permettrait une amélioration marquante de la capacité à détecter et à identifier les agents d'origine biologique.

Plans futurs: On prévoit d'étendre cette étude à d'autres types de poudres et sols ainsi qu'à d'autres agents représentant une menace biologique.

Table of contents

Abstract	i
Résumé	i
Executive summary	iii
Sommaire	iv
Table of contents	v
List of figures	vi
List of tables	vi
Acknowledgements	vii
1 Introduction.....	1
2 Material and methods.....	2
2.1 MIM Analyzer	2
2.2 Reagents and Materials.....	2
2.3 Assays.....	3
2.4 Antigens.....	3
2.5 Laboratory Protocol.....	3
2.6 Field Protocol	3
2.7 Analysis of Data and Statistics	4
3 Results and Discussion	5
4 Conclusion	11
References	12
Annex A Running a Plate on the MIM.....	15
Annex B Powder Swab Protocol	17
Annex C Soil Swab Protocol	18

List of figures

Figure 1. MIM assay of <i>F. tularensis</i> in powders and soil using the BioVeris suggested matrix:diluent of 1:10.....	6
Figure 2. MIM assay using ricin reagents: increasing ratios of BV-DILUENT™ to matrices.....	7
Figure 3. MIM assay of ricin using the final protocol for the use of swabs to assay powders and soil	9

List of tables

Table 1. Amounts of powders on swabs.....	8
Table 2. MIM assay of dairy creamer, flour, and soil from swabs	10
Table 3. MIM assays of flour and soil in triplicate.....	10

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1 Introduction

Incidents involving suspicious powders, especially those involving anthrax and ricin-laced letters in the US postal system [1], have led to substantial effort at DRDC Suffield and elsewhere to develop suitable technologies and sampling procedures for the analysis of such materials.

Some work on the development of methods to detect bacteria in powders has been reported for *Bacillus thuringiensis* and other viable spores [2, 3]. Successful use of a fluorescence-based hand-held assay system, the Rapid Analyte Measurement Platform (RAMPTM), has been described for detection of ricin in a variety of common powders [4]. The Association of Analytical Communities (AOAC) International [5] has developed and approved a standard procedure for collecting, packaging, and transport of samples of visible powders suspected of being biological threat agents [6]. A review of current and developing technologies for monitoring biological threat agents includes methods for sample processing of a variety of matrices including powders and soil [7].

Much has been published on the analysis of a variety of substances in soil ranging from inorganic metals [8–10], to pesticides [11], herbicides [12], polycyclic aromatic hydrocarbons [13], and other pollutants [14, 15]. A common thread in most of these publications is the necessity for clean-up step(s) prior to assay, to prevent interfering components of the soil from generating false positives [8–10]. For biological agents, the usual method of detection is to dilute the soil sample and isolate the agent in suitable media [16]. While this technique may be feasible for identification of live agents, it is not suitable for non-living organisms or toxins. Successful use of an artificial marine worm gut fluid has been described for the extraction of *Bacillus thuringiensis* from soil [17]; however, little analytical data is available on the extraction of biothreat agents from soil prior to analysis. In-house experimentation with soil clean-up for subsequent analysis by ELISA has resulted in little success in removing background interferents*.

Electrochemiluminescence (ECL) is a relatively new technology for conducting immunoassays with improved assay performance [18, 19]. The advantages of ECL over enzyme-linked immunosorbant assay (ELISA) and many other immunoassays include low detection limits (high sensitivity) due to high luminescent signal-to-noise ratios [20, 21], large dynamic range of analyte detection of approximately five orders of magnitude [21, 22], reduced labour intensity, and short assay time (16 minutes, including incubation).

ECL assays have been reported for a number of biological threat agents including *Staphylococcus enterotoxin B*, ricin, *Vibrio cholerae*, Botulinum toxin, and *Bacillus anthracis* [21, 23, 24]. ECL detection of biological agents in both food and environmental samples has also been studied [25].

The M1M (BioVeris Corp., Gaithersburg, MD), an automated electrochemiluminescent (ECL) assay instrument, is currently being considered by several agencies for field identification of biothreat agents. The United States Critical Reagents Program (CRP) has developed ECL M1M assays for nine high priority biothreat agents and these are available, off-the-shelf, to US and allied military forces and first responders. ECL M1M technology is used in defence laboratories in the United States, United Kingdom, and Canada; and has been successfully fielded by several

* Unpublished results.

Theatre Labs of the US Army and Air Force. The M1M technology has been shown to be robust and tolerant of samples in a variety of complex matrices; however, methods for sampling of powders and soils in the field for processing on the M1M have not been described.

This report describes the development of simple and reproducible methods that may be used for field sampling of powders and soils for subsequent screening and analysis on the M1M. To acquire the sample, polyester swabs were chosen, as they were deemed the best option for dealing with powders and soils on a variety of surfaces. A procedure for “picking up” reproducible amounts of powder or soil was devised and standardized. An average amount of powder or soil collected on the swab when using the standard method was determined to be 12 mg. Using this value, the volume of diluent required to produce a signal <1.2 on the M1M was empirically determined. The powder or soil suspension was filtered and the filtrate assayed on the M1M. The result was a method that could be used consistently to assay powder and soil by ECL M1M with good sensitivity and minimal background signal.

2 Material and methods

2.1 M1M Analyzer

An M-SERIES^R M1MR ECL Analyzer was purchased from BioVeris Corp. (Gaithersburg, MD) and operated in the M1M mode for these experiments.

The BioVeris M-SERIES^R M1M Analyzer is an automated single channel system based on ECL technology. The components of the ECL immunoassay are biotinylated capture antibody bound to streptavidin-coated magnetic beads; the antibody-bead complex binds antigen and is drawn into the ECL-analyzer flow cell; detector antibody, labelled with ruthenium-trisbipyridal, for the emission of light when electrochemically stimulated; and a precursor molecule, tripropylamine, which is activated on an electrode surface, resulting in an electron transfer reaction that initiates excitation of the ruthenium-trisbipyridal, resulting in the emission of light at 620 nm. This excitation/emission cycle repeats many times, resulting in increased signal from a single binding event, resulting in increased sensitivity [18].

The complete steps for running an assay on the M1M analyzer were abbreviated from the BioVeris Operation Manual, and are presented in Annex A.

2.2 Reagents and Materials

The following M-SERIES^R reagents were purchased from BioVeris Corp: BV-CLEANTM Plus Solution, BV-GLOTM Plus Solution, BV-STORETM Solution, BV-DILUENTTM Solution, BV-SANITIZETM Solution, M-SERIES^R Positive Calibrator, and M-SERIES^R Negative Calibrator.

BV Sample Preparation Filters, 60 µm, catalogue number 110117, were purchased from BioVeris Corp. Dacron^R Polyester Tipped Swabs, Fisherbrand^R, catalogue number 14-959-90, were purchased from Fisher Scientific (Ottawa, ON).

The following powders were purchased from the US CRP: baking soda, lot number DPG1DEC03; dairy creamer, lot number DPG13MAY04; and flour, lot number DPGLJ24MAY05. In addition, flour (white, enriched, all purpose, Safeway brand) and dairy creamer (Coffee Mate[®], Carnation, Nestlé) were purchased from Canada Safeway Ltd. (Medicine Hat, AB). The soil was from the Suffield Experimental Proving Ground (Suffield EPG) and had been characterized by Alberta Environmental Centre (Mr. P. Yeung, Vegreville, Alberta) as sandy clay loam.

2.3 Assays

MINITube assays for *Francisella tularensis*, ricin, and Venezuelan equine encephalitis virus (VEEV) were purchased from the US CRP. MINITube assays incorporate lyophilized reagents required to perform the MIM assays, i.e. biotinylated anti-analyte antibody bound to streptavidin-coated magnetic beads and ruthenium-labelled anti-analyte antibody. Positive control medium (PCM) and positive control low (PCL) antigens for these agents (inactivated) were also purchased from the US CRP.

2.4 Antigens

The following ⁶⁰Co-irradiated antigens were purchased from the US CRP: *Francisella tularensis* (Schu 4), lot number AGD0000541, stock concentration 3.1×10^9 cfu/mL; and VEEV (TC83), lot number AGD0000109, stock concentration 1.1×10^{10} pfu/mL. Ricin (RCA₆₀), catalogue no. L8508, lot no. 63H4026, was purchased from Sigma-Aldrich Canada (Oakville, ON). Prior to use, the stock ricin was diluted in phosphate buffered saline (PBS), pH 7.4, to a working stock concentration of 390 µg/mL and stored at 4°C.

2.5 Laboratory Protocol

A standard procedure recommended by BioVeris, in which powder and soil were prepared at concentrations of 100 mg/mL in BV-DILUENT[™] was initially evaluated. Baking soda (2 x 100 mg), CRP flour (2 x 100 mg), and soil (2 x 100 mg) were weighed into each of six 5 mL polystyrene tubes (Fisher Scientific). BV-DILUENT[™] (1 mL) was added to each tube. To one tube each of the baking soda, flour, and soil mixtures was added 1.61 µL *F. tularensis* stock to yield final concentrations of 5×10^6 cfu/mL. The two baking soda solutions (*F. tularensis*-spiked and unspiked) were assayed without further sample preparation, according to the general MIM procedure described in Annex A. The flour and soil mixtures (spiked and unspiked) were filtered through BV Sample Preparation Filter tubes and aliquots of the filtrates assayed, as above.

2.6 Field Protocol

Samples of CRP dairy creamer (4 x 200 mg), CRP flour (4 x 200 mg), and soil (4 x 1g) were weighed into each of twelve 5 mL polystyrene tubes (Fisher Scientific). Ricin was prepared in nanopure water at a concentration of 833 ng/mL for use with powder (creamers and flour) and 2.08 µg/mL for use with soil. One mL ricin solution at 833 ng/mL was added to each of the eight tubes containing the powders. Two mL ricin solution at 2.08 µg/mL was added to each of the four tubes

containing soil. All tubes were vortexed for 15 seconds and their contents poured into individual plastic weigh boats (1 inch square) (Fisher Scientific). The weigh boats containing the ricin-spiked powders and soil were left to air dry in a Class II Biosafety Cabinet for 72 hours. Each dried material was crushed in a mortar with pestle and placed in a microfuge tube for later use.

In order to determine if a consistent amount of powder or soil could be picked up by a swab from a small sample of matrix, the following protocol was followed. A swab was wet with BV-DILUENT™ and then pressed against the sides of the tube containing the buffer to remove excess liquid from the swab. The swab was then weighed on a five decimal place balance. The wet swab was rolled in the powder or soil to acquire maximum sample and then tapped vigorously to remove excess matrix, then weighed again. Five replicates of each of dairy creamer, flour, and soil were performed by one individual, while five replicates of flour were performed by a second individual who followed a written protocol and was not further coached as to procedure.

Increasing amounts of BV-DILUENT™ were used in assaying the powders and soils coated on swabs until all unspiked matrices yielded signals that were well below the S/B cut-off of 1.2, while positive signals from spiked matrices were maintained. The final assay protocol was verified for reproducibility on the MIM by evaluating triplicate samples of both unspiked and ricin-spiked flour and soil. A shortage of CRP ricin MINITubes precluded the inclusion of dairy creamer in these final assays. Further verification of optimum matrix to diluent ratios was obtained by using VEEV MINITubes and positive control antigens with flour from a different source (Safeway brand) and soil samples. In these experiments, performed in triplicate, VEEV, was manually added to swabbed flour in 1mL BV-DILUENT™ and swabbed soil in 2 mL BV-DILUENT™, to yield a VEEV concentration of 1×10^5 pfu/mL. The final protocols for use in powder and soil sampling for subsequent analysis on the MIM are attached in Annexes B and C, respectively.

2.7 Analysis of Data and Statistics

Negative (no antigen) controls were tested in replicates of four (two prior to matrix assay and two at conclusion of matrix assay). Positive control antigens (PCL and PCM) were tested in replicates of two tubes/assay. Unless otherwise noted, all other samples were tested singly.

ECL readings were considered positive if the emission reading was significantly greater than the background emission reading. Statistical significance, pre-programmed into the MIM, was established at 20% above background (no antigen control). Therefore a signal was considered positive if the value of the sample signal to background signal (S/B) was 1.2 or greater. An automated alarm sounded if any one of the signals had a S/B greater than 1.2.

Data was manually exported from the MIM to Microsoft Excel 7.0 and was graphed using Grapher 6.0 (Golden Software Inc., Golden, CO).

3 Results and Discussion

Soil has proven problematic as a sample matrix in immunoassays, particularly ELISAs, due to the presence of background interferents generating false positive signals. Non-specific positive reactions were observed when aqueous solutions of soil (clay, loam, or sand) were used to challenge agent-specific ELISAs as potential assay interferents [26]. ELISAs of *Bacillus anthracis*, *Francisella tularensis*, and ricin performed on soil that had been sonicated and treated with a variety of extracting media including PBS, PBS/2% BSA, PBS/2% BSA/0.1 % Tween 20, PBS/0.5% BSA/0.1% SDS, and PBS/0.5% BSA/0.1% sodium taurocholate [17] all produced high backgrounds*. The only success in detecting ricin in soil by ELISA was by the use of solid phase extraction methods in the treatment of soil collected from a remedial waste site at DRDC Ottawa. However, this soil clean-up method was time-consuming and complicated and recovery of the ricin was low (15–20%)*.

The results for the protocol recommended by BioVeris Corp. (100mg powder/1 mL BV diluent) are graphically represented in Figure 1. MIM assays for baking soda, flour, and soil, both unspiked and spiked with *F. tularensis*, were all positive. BioVeris Corp. recommends a ratio of sample matrix to diluent of 1:10 (1 mg/10 μ L = 100 mg/mL) for the analysis of powders and soils. However, BioVeris Corp. uses a Signal/Background (S/B) cut-off of 2.0 for their MIM assays, while the CRP MINITube kits effect a software adjustment of S/B = 1.2. Therefore, it was not unexpected that with the more stringent S/B cut-off being set at 1.2, false positive signals occurred. The observation that false positives occurred, however, indicated that more diluent would need to be added to decrease the background signal.

Depletion of the *F. tularensis* MINITubes early in the study dictated that MINITubes for a different agent be used for subsequent experiments; hence ricin MINITubes and antigen were selected for ensuing studies. In order to estimate the approximate ratio of powder and soil to diluent necessary to avoid false positives, a series of ratios of creamer, flour, and soil (unspiked) to diluent were assessed on the MIM: 100 mg/1 mL; 100 mg/2 mL; 100 mg/3 mL; 100 mg/4 mL; 100 mg/5 mL. The results of this experiment are graphically represented in Figure 2. Both powders (dairy creamer and flour) produced S/B signals that were below the S/B cut-off of 1.2. The soil, however, produced signals that were above the S/B 1.2 cut-off (false positives) for all five test samples examined, thus indicating that additional diluent was warranted. From this experiment, it was deemed that, while the powders could be used at the powder to diluent ratio tested, the soil samples would require that additional diluent be added.

The results of experiments to determine the average amount of material coated on a swab when following a standardized swabbing procedure are presented in Table 1. From the data, the average amount of powder “picked up” by a swabbing procedure was 12 mg. This weight represents the mid range between the median (10.9 mg) and the mean (12.89 mg) amount of powder deposited on a swab by two different individuals following the same swabbing procedure. An estimate of the amount of soil deposited on a swab when the same standard sampling procedure was followed was impossible to determine, as wet swabs coated with soil actually weighed less than the wet swabs themselves. This could have been due to the wicking of BV-

* Unpublished results.

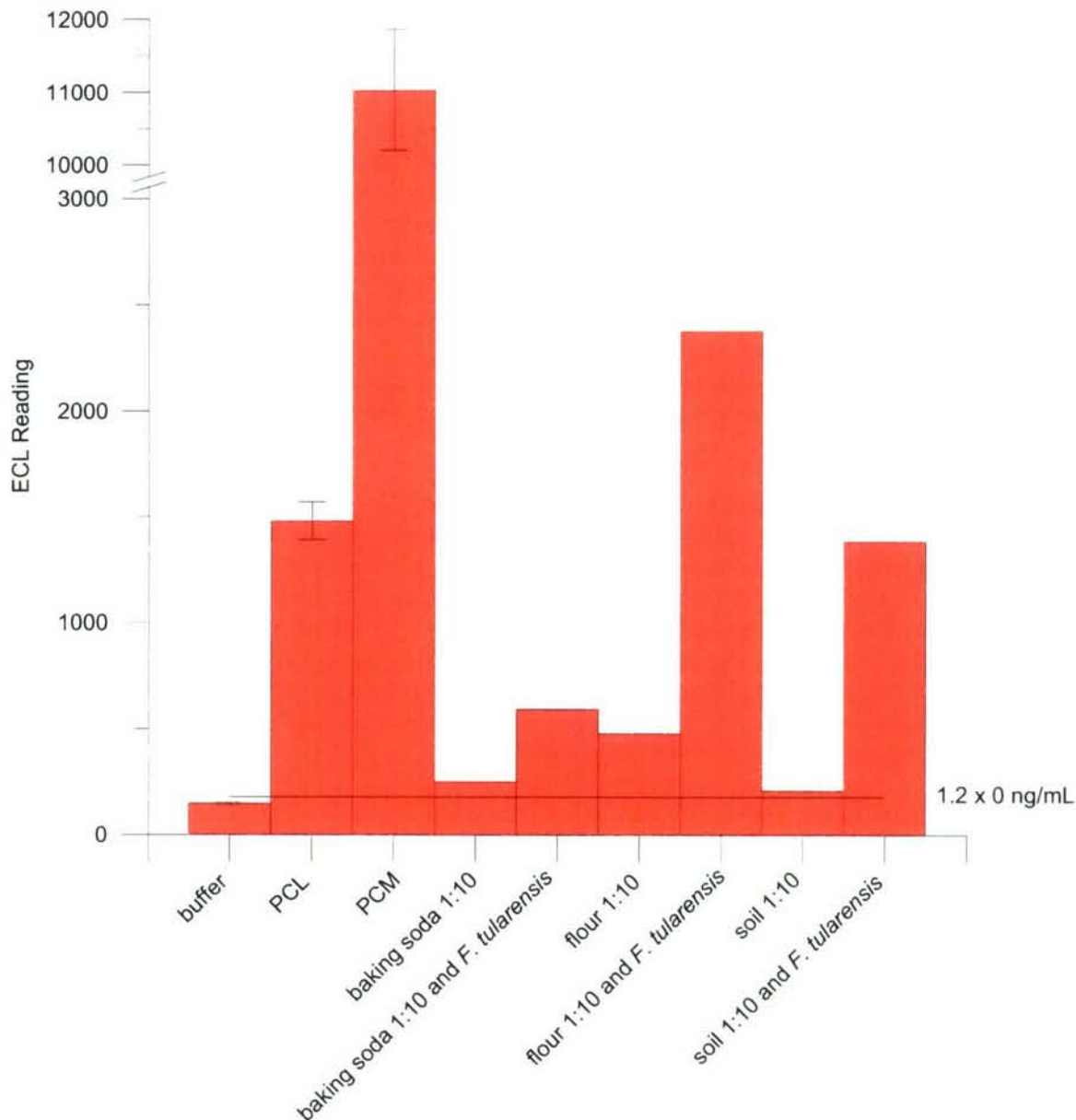


Figure 1. MIM assay of *F. tularensis* in powders and soil using the BioVeris suggested matrix:diluent of 1:10

DILUENT™ from the swab to the dry soil, producing localized muddy sections of soil in the weigh boats. Further experiments are required to estimate the average weight of soil “picked up” by a wet swab, but for the purposes of this study, the average weight for powders (12 mg) was also used for soil ratio calculations.

The next step in this study was to transition procedures from the laboratory method of weighing soils and powder to a method using swabs that would be suitable for use by operators in the field.

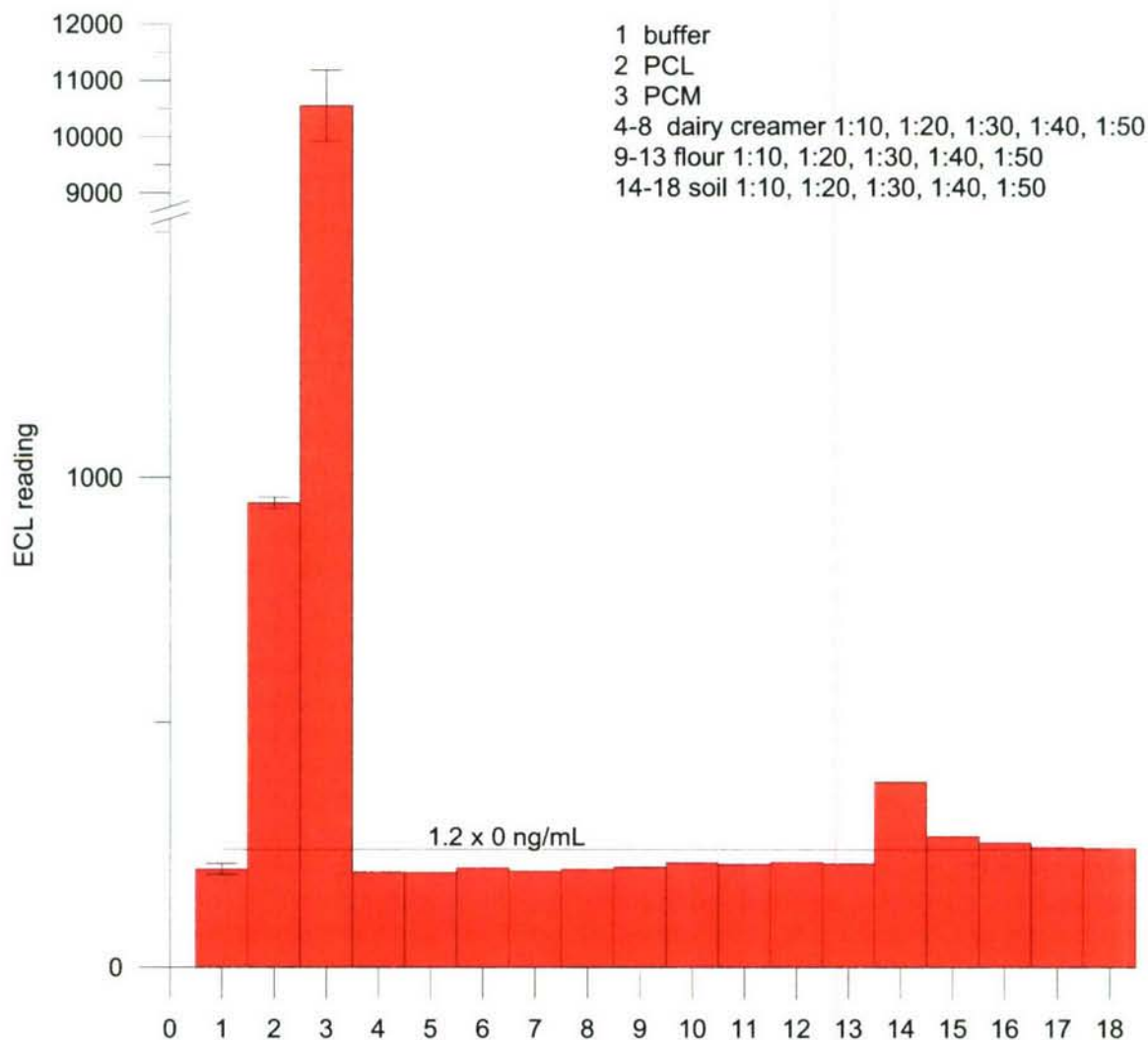


Figure 2. MIM assay using ricin reagents: increasing ratios of BV-DILUENT™ to matrices

Based on the results obtained using weighed amounts of unspiked powder and soil, ratios of ricin-spiked matrix to diluent starting at 1:20 (12 mg/240 µL) were used for creamer and flour. Although a ratio of 1 part matrix to 10 parts diluent had not resulted in a false positive when using weighed amounts of matrix (Figure 2), this ratio had resulted in the generation of a false positive in the initial BioVeris protocol experiment using *F. tularensis* reagents (Figure 1). Thus, ratios of 1:20 (12 mg matrix/240 µL diluent), 1:30 (12 mg matrix/360 µL diluent), and 1:42 (12 mg matrix/ 500 µL diluent) were tried for the creamer and flour swabs. No false positives were observed for the dairy creamer at any of the ratios examined (data not shown). Complications in the form of plugged filters were encountered in the filtration of the flour samples at lower matrix to diluent ratios (1:20 and 1:30); therefore additional BV-DILUENT™ was required to be added to the Sample Prep Filter tubes to generate sufficient flour filtrate (50 µL) for analysis. In spite of increasing the matrix to diluent ratio to 1:42, the signal for unspiked flour was above the S/B cut-off of 1.2, indicating that even further diluent was necessary.

Table 1. Amounts of powders on swabs

Powder	Individual 1			Individual 2		
	Average weight (mg)	Standard Deviation	CV (%)	Average weight (mg)	Standard Deviation	CV (%)
creamer	15.7	3.4	21	-	-	-
flour	12.5	4.0	32	10.8	2.3	21

When soil was used as matrix, all the soil to diluent ratios examined (1:10–1:50) resulted in false positives (Figure 2). Therefore, a starting ratio of one part soil to 80 parts diluent (1:80), 1:100, and 1:125 were tried. For all of these ratios of soil to diluent, no false positives were generated (data not shown). However, the S/B ratios for all unspiked soil assays were very close to the 1.2 assay cut-off i.e., S/B=1.177, 1.198, and 1.196 for matrix to diluent ratios of 1:80, 1:100, and 1:125, respectively. Hence, the addition of further diluent was considered prudent.

A final protocol for sampling of ricin-spiked powders and soils with polyester swabs was arrived at by selecting a matrix to diluent ratio at which all unspiked matrices produced signals that were acceptably below the 20% above background cut-off (S/B=1.2), while still giving a positive signal for ricin-spiked matrices. Thus, a matrix to diluent ratio of 1:83 (12 mg/1 mL) was selected for powders and 1:167 (12 mg/2 mL) was selected for soils. Results indicated that all unspiked matrices produced signals acceptably below S/B=1.2, while all ricin-spiked matrices were positive (Figure 3; Table 2). Experiments to confirm these findings were conducted using unspiked and ricin-spiked flour and soil matrices in triplicate assays (Table 3). All unspiked matrices resulted in signals well below the 1.2 cut-off, while the ricin-spiked matrices generated positive results. The creamer was not assayed as there were insufficient ricin MINITubes to complete the desired replicates. However, dairy creamer had not posed a problem with false positives from the beginning of the study, and this omission was considered acceptable.

Further verification of the optimum matrix to diluent ratio was obtained using VEEV MINITubes, antigens, and matrices more commonly used at DRDC Suffield. For this verification, replicates of three assays of each of flour (Safeway brand), coffee creamer (Nestlé brand), and soil, both unspiked and spiked, with VEEV (5×10^5 pfu/mL) were tested. Results indicated that no false positives were generated in matrix only assays. Although VEEV-spiked matrices also did not yield positive results, this was attributed to the later finding that VEEV at 5×10^5 pfu/mL was considerably below the LOD of the MIM (data not shown). Due to time constraints imposed by the deployment schedule and depletion of available VEEV MINITubes, it was not possible to repeat these assays using spiked VEEV soil at higher concentration. In follow-on experiments, the LOD of VEEV on the MIM was determined to be approximately 5×10^7 pfu/mL (data not shown); hence a concentration of 1×10^9 pfu/mL was recommended for future use of VEEV.

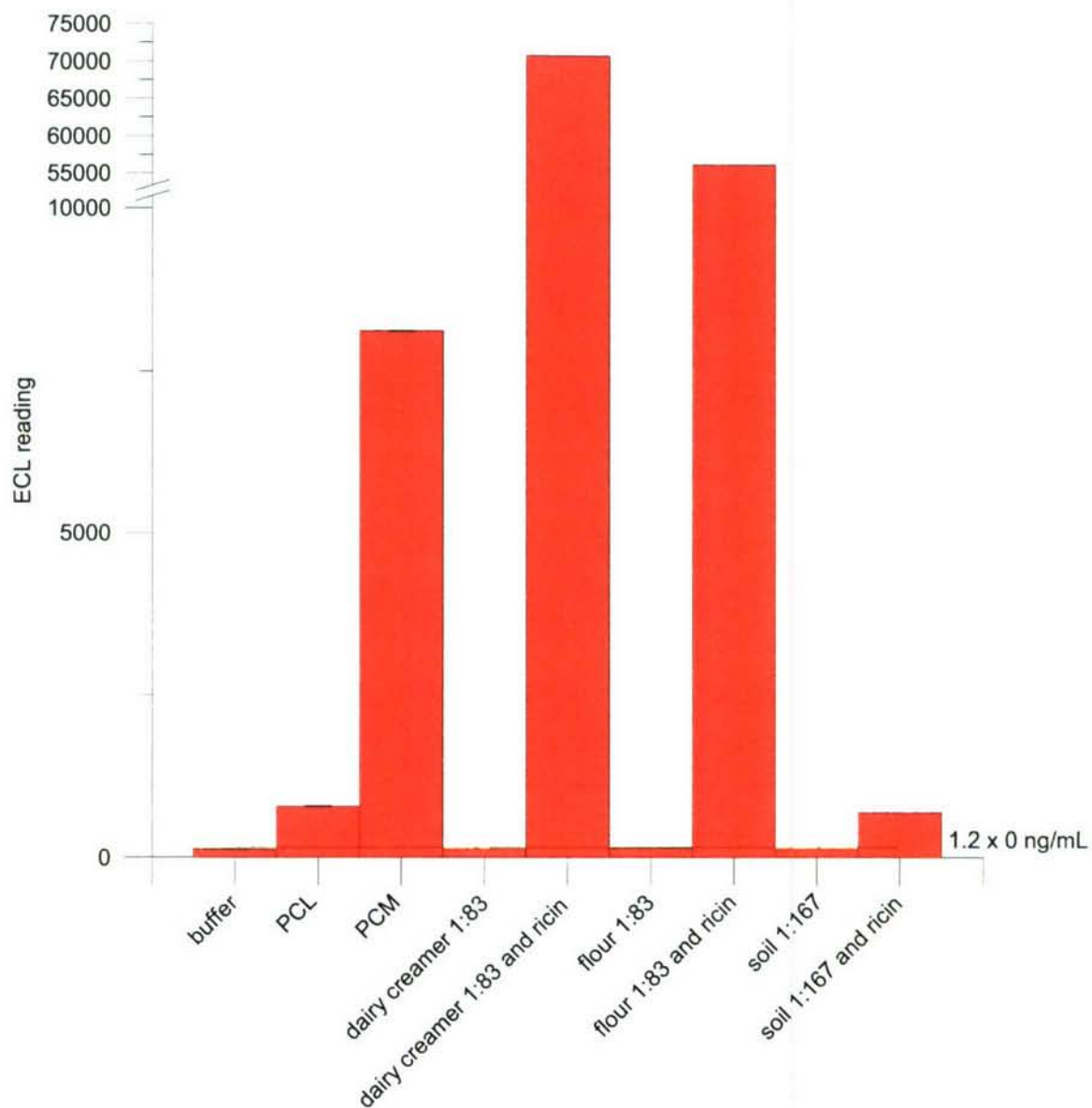


Figure 3. MIM assay of ricin using the final protocol for the use of swabs to assay powders and soil

Table 2. MIM assay of dairy creamer, flour, and soil from swabs

Sample	Signal	Standard Deviation	Signal/Background
buffer	132	3.9	-
PCL	780	7.9	5.909
PCM	8123	12.6	61.538
creamer 1:83	141		1.068
creamer and ricin 1:83	70760		536.061
flour 1:83	147		1.114
flour and ricin 1:83	56046		424.591
soil 1:167	141		1.068
soil and ricin 1:167	690		5.227

Table 3. MIM assays of flour and soil in triplicate

Sample	Signal	Standard Deviation	Signal/Background
buffer	128	1.8	-
PCL	984	11.1	7.688
PCM	10423	271	81.430
flour 1:83	135	0.58	1.055
flour and ricin 1:83	66447	10327	519.117
soil 1:167	140	7.8	1.094
soil and ricin 1:167	435	106.4	3.399

4 Conclusion

A procedure for “picking up” reproducible amounts of powder and soil on swabs for subsequent ECL analysis on the M1M platform was devised and standardized for use in the field. The ECL M1M technology is sensitive in detection of biological agents in a variety of complex matrices, such as soil and powders, likely to be encountered by the CF and First Responders. Both the sampling protocol developed here and the operation of the M1M are rapid and relatively simple and easily transferable from a laboratory setting to the field. The study will be expanded to include other powders and soil types as well as other biothreat agents.

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Annex A Running a Plate on the M1M

1. Turn on the M1M analyzer
2. Turn on the computer and log into Windows. The M-Series M1M analyzer software loads and displays automatically. If it doesn't, double click on the M1M icon on the desktop.
3. Click **New Plate** from the *M-Series M1M Analyzer* window.
4. Enter a title in the *Plate Name* box of the *New Plate Info* box.
5. Click **Next Step** in the bottom right of the window.
6. Click on **Test Kit ID** field. Scan the first Bar Code (A) of the CRP kit package and the box will fill with a code.
7. Click on **Sample ID** field. Enter a sample name such as "unknown no. 1".
8. If doing more than one replicate of each sample, click on **Number of Replicates** and enter number of replicates being tested.
9. Click on **Next Sample**.
10. Enter the second sample name in the **Sample ID** field.
11. When all samples have been entered, click **Next Step** in the bottom right of the window. *Control Lot #* window is displayed.
12. Click **OK**. *Final Template* window is displayed.
13. Check to see if as desired. If not, back up a step. If OK, click **Done**. The *Run a Plate* window is displayed
14. Verify that both the "Plate Summary" and the "Samples & Controls" information are correct. To make corrections, highlight a test in the *Samples & Controls* area and use **Add**, **Edit**, or **Delete**.

Sample Preparation

- a. Reconstitute PCM and PCL with 250 μ L of BV-DILUENT to each vial
- b. Open bar-coded package containing tubes of lyophilized material.
- c. Place tubes in Tube Rack according to plate template.
- d. Add 50 μ L BV-DILUENT to negative control (NC) wells.

- e. Add 50 μ L of CRP Positive Control Medium (PCM) to specified wells.
 - f. Add 50 μ L of CRP Positive Control Low (PCL) to specified wells.
 - g. Add 50 μ L of sample (for example: unknown no. 1) to test wells.
 - h. Add 50 μ L BV-DILUENT to all wells being used.
15. Place the prepared plate in the plate tray with well A1 towards the back of the MIM analyzer.
 16. Click **Run** on the toolbar to start the plate reading process. As results are calculated, they are displayed in the *Plate Layout* area.
 17. Check the plate status at the bottom of the *Plate Layout* area to track progress of the plate read.
 18. Click **Eject** from the toolbar to eject the plate tray when the plate read is completed

Annex B Powder Swab Protocol

1. Remove swab from wrapping
2. Dip swab in vial or tube of BV-DILUENT and thoroughly wet swab
3. Remove swab from liquid and press swab against sides of tube to remove excess liquid
4. Roll swab in powder to thoroughly coat swab
5. Hold swab between thumb and middle finger and use index finger to firmly tap stem of swab to remove excess powder (as though flicking the ash from a cigarette).
6. Dip coated swab in tube containing 1 mL BV-DILUENT, manually agitating swab in the liquid for about 10 seconds.
7. Remove swab from liquid and press swab against sides of tube to remove excess liquid.
8. Thoroughly mix powder in liquid by shaking or vortexing tube, and pour or pipette contents of tube into the BioVeris Sample Prep Filter.
9. Remove bottom cap of Sample Prep Filter and repeatedly squeeze below filter disk to empty filtered fluid into a clean tube. At least 50 μ L is needed to run an assay. Filtered liquid may be cloudy.
10. Proceed with assay as described in the manual
11. Soil Swab Protocol assay as described in the manual

Annex C Soil Swab Protocol

1. Remove swab from wrapping
2. Dip swab in vial or tube of BV-DILUENT and thoroughly wet swab
3. Remove swab from liquid and press swab against sides of tube to remove excess liquid
4. Roll swab in soil to thoroughly coat swab
5. Hold swab between thumb and middle finger and use index finger to firmly tap stem of swab to remove excess soil (as though flicking the ash from a cigarette).
6. Dip coated swab in tube containing 2 mL BV-DILUENT, manually agitating swab in the liquid for about 10 seconds.
7. Remove swab from liquid and press swab against sides of tube to remove excess liquid.
8. Thoroughly mix soil in liquid by shaking or vortexing tube, and pour or pipette contents of tube into the BioVeris Sample Prep Filter.
9. Remove bottom cap of Sample Prep Filter and repeatedly squeeze below filter disk to empty filtered fluid into a clean tube. At least 50 μ L is needed to run an assay. Filtered liquid may be cloudy.
10. Proceed with assay as described in the manual

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The M1M (BioVeris Corporation), an automated electrochemiluminescent (ECL) assay instrument, has been shown to be a technology that is robust and tolerant of samples in a variety of complex matrices; however, methods for sampling of powders and soils in the field for processing on the M1M have not been described. This report describes the development of simple and reproducible methods that may be used for field sampling of powders and soils for subsequent screening and analysis on the M1M. By sampling with polyester swabs using a standard method, it was found that it was possible to collect a reproducible amount (~12 mg) of powder or soil from a variety of surfaces. Using this value, the volume of diluent required to produce a signal <1.2 on the M1M was empirically determined. The powder or soil suspension was filtered and the filtrate assayed on the M1M. The result was a method that could be used consistently to assay powder and soil by ECL M1M with good sensitivity and minimal background signal.

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