Review and analysis of bioidentification systems for mobile laboratory and field use

William Lee
DRDC – Suffield Research Centre

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Abstract

The present report is a review and analysis of the operational performance of mobile laboratory and field-use identification systems for biological threat agents. The main source for the review and analysis was the *Edgewood Biosensors Test Bed Hand-held and Man-portable Edition* (2013) plus market surveys of commercial systems published by US Department of Defense (2011 and 2014). The instruments or systems evaluated in the *Edgewood Biosensors Test Bed* and the surveys were commercial off-the-shelf instruments or prototypes at an advanced stage of development. The focus of the *Edgewood Biosensors Test Bed* was the performance of the instruments in the hands of laboratory scientists and trained military field operators. For the hands-on evaluation 11 systems based on nucleic acid (polymerase chain reaction) or antibody-based (immunoassay) technology were downselected. The *Edgewood Biosensors Test Bed* showed the importance of conducting the evaluations in a real-world setting, i.e., actually taking field-use instruments to the field with military operators. The purpose of the present report is to provide project managers in the Canadian Armed Forces (CAF) with an outline for making decisions on acquisition of bioidentification instruments and systems. The report also provides guidance for project managers on how to conduct evaluations of candidate instruments. Because of the varied operations of CAF personnel, no single instrument will likely meet the requirements of all deployments. Several of the highly rated instruments in the *Edgewood Biosensors Test Bed* are potential candidates for use by the CAF. The main conclusion of the report is that assessment of bioidentification instruments for CAF operations is a difficult but tractable problem. The report contains a strategy to dissect the problem and achieve meaningful knowledge that can be applied to a procurement process.

Significance to defence and security

Mobile laboratory and field-use bioidentification systems are requirements for the Canadian Armed Forces (CAF) in order to support operations at home and abroad. In 2016 a limited number of commercial-off-the-shelf (COTS) systems were available but none likely possessed all the attributes needed for CAF procurement. However several of the highly rated COTS systems that were evaluated by the *Edgewood Biosensors Test Bed Hand-held and Man-portable Edition* (2013) would likely be suitable with modifications for military use. For a major procurement of bioidentification equipment, market surveys based on vendor-supplied specifications, test data and information will not be sufficient for making procurement decisions. Actual hands-on assessments are required in real-world environments by CAF end-users and by the support networks in Defence Research and Development Canada. The significance of the report is the dissection of the problems associated with the assessment of bioidentification instruments for procurement into resolvable units. The present report provides information and guidance for project managers on how to conduct evaluations of candidate systems. The overall process of mobile and field bioidentification has four components: 1) system hardware, 2) sample preparation, 3) assay design and reagents, 4) operator interface and performance. Although procurement will address the first component, namely, the bioidentification system, consideration of the other three components prior to and during decision-making for procurement is necessary for the success of what would be procured.

Résumé

Le présent rapport porte sur l'examen et l'analyse de la performance opérationnelle des laboratoires mobiles et des systèmes d'identification sur le terrain des agents de menace biologique. Pour les besoins de l'examen et de l'analyse, nous avons utilisé principalement le modèle portatif du banc d'essai de biocapteurs Edgewood (2013), ainsi que des études de marché sur des systèmes commerciaux réalisées par le département de la Défense des États-Unis (2011 et 2014). Les systèmes évalués avec le banc d'essai de biocapteurs Edgewood et les études de marché étaient des instruments ou des prototypes commerciaux à un stade avancé de développement. Nous avons utilisé le banc d'essai dans le but d'évaluer la performance des instruments entre les mains de chercheurs de laboratoire et d'utilisateurs militaires exercés sur le terrain. Pour l'évaluation pratique, nous avons sélectionné 11 systèmes fondés sur une technologie à base d'acide nucléique (réaction en chaîne de la polymérase) ou d'anticorps (immuno-essai). L'utilisation du banc d'essai de biocapteurs Edgewood a révélé l'importance de mener les essais en situation réelle, c'est-à-dire en demandant à des utilisateurs militaires d'utiliser les instruments sur le terrain. Le présent rapport a pour but de fournir aux gestionnaires de projets des Forces armées canadiennes (FAC) un cadre de prise de décision concernant l'acquisition d'instruments et de systèmes de bioidentification. Il fournit également aux gestionnaires de projets des lignes directrices pour l'évaluation des instruments d'intérêt potentiel. En raison de la nature variée des opérations des FAC, il est probable qu'aucun instrument ne réponde aux besoins de tous les déploiements. Plusieurs des instruments les mieux cotés par le banc d'essai des biocapteurs Edgewood sont susceptibles d'être utilisés par les FAC. La conclusion principale du rapport est que l'évaluation d'instruments de bioidentification destinés aux opérations des FAC est un problème complexe mais soluble. Le rapport propose une stratégie pour disséquer ce problème et ainsi acquérir un savoir considérable et applicable aux processus d'approvisionnement.

Importance pour la défense et la sécurité

Les Forces armées canadiennes (FAC) ont besoin de laboratoires mobiles et de systèmes de bioidentification sur le terrain pour assurer le soutien de leurs opérations menées au pays comme à l'étranger. En 2016, un nombre limité de systèmes commerciaux étaient offerts, mais aucun ne semblait posséder toutes les caractéristiques nécessaires pour inciter les FAC à s'en procurer. Cependant, plusieurs des instruments commerciaux les mieux cotés par le modèle portatif du banc d'essai de biocapteurs Edgewood (2013) conviendraient probablement si on y apportait des modifications à des fins militaires. Par contre, pour ce qui est de procéder à un achat majeur d'équipement de bioidentification, les études de marché fondées sur des caractéristiques techniques, de données d'essais et d'informations obtenues auprès de fournisseurs ne suffisent pas pour prendre une telle décision. C'est pourquoi il est nécessaire de réaliser des essais pratiques en situation réelle exécutés par les utilisateurs finaux des FAC et les réseaux de soutien de Recherche et développement pour la défense Canada. L'importance du présent rapport se reflète dans la dissection des problèmes associés à l'évaluation des instruments de bioidentification aux fins d'achat en unités solubles. Il fournit de l'information et des lignes directrices à l'intention des gestionnaires de projets sur la façon d'évaluer les systèmes candidats. Le processus d'évaluation

des systèmes de bioidentification mobiles/sur le terrain comporte quatre éléments : 1) matériel du système; 2) préparation des échantillons; 3) conception de l’essai et réactifs; 4) interface utilisateur et performance. Bien que le premier élément (en l’occurrence, le système de bioidentification) est évalué pendant le processus d’achat, il est nécessaire d’évaluer les trois autres éléments avant et pendant le processus décisionnel relatif à l’achat pour assurer un choix judicieux.

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1 Introduction

The threat of dissemination of biological agents creates a problem for the Canadian Armed Forces (CAF). The response to a biological agent incident will be dependent upon the location, prior and immediate knowledge, and the role of the responders. Clearly biodetection/identification/monitoring (BioDIM) systems are required to guide decision-making in order to manage the threat effectively. The BioDIM requirements of responders from various units might be somewhat different based on their missions but they will all need specific and time-sensitive information for making decisions about the situation at hand. Because of the wide-ranging operations that CAF personnel undertake, no single instrument will likely fulfill all operational requirements. However there will be situations where requirements may overlap and the same system(s) may serve more than one operation, hence reducing the number of procurements. A review and analysis of commercial (and military) off-the-shelf (COTS and MOTS) bioanalytical instruments is presented here. This report invokes the concept of the "test bed": a platform for conducting rigorous, transparent, and replicable testing of new technologies, scientific theories, and computational tools. The concept is employed by many disciplines to describe experiments to assess the performance of new technologies in specific and defined environments [1].

The sources for the present scientific report were the *Chemical Biological Radiological Technology Survey (CBRTS)* (2011) [2], the *Global CBRN Detector Market Survey* (2014) (GDMS) [3] and the *Edgewood Biosensor Test Bed Hand-held and Man-portable Edition* (2013) (BTB) [4]. The present report contains analysis and recommendations based on input from Defence Research and Development Canada (DRDC) subject matter experts (SMEs). The surveys (2011 and 2014) were large documents, hundreds of pages each, covering hundreds of analysis instruments and systems. The products listed in the surveys were commercially available or were models having high technology readiness levels (TRLs). The survey data was derived from vendor-supplied information and specifications in response to an extensive questionnaire. Each instrument was scored against specific criteria pertaining to the scenario of use. Overall scores and rankings were generated using statistical packages for social sciences. The GDMS (2014) and the BTB (2013) were close in time so the candidate pools of detection systems were similar. GDMS covered biological, chemical, radiological detector systems and evaluated according to four scenarios of deployment: field use, mobile laboratory, diagnostic laboratory, and high-sensitivity high-throughput analytical laboratory. The GDMS did not differentiate amongst underlying methods of analysis such as nucleic acid, antibody-based or aerosol particle detection. The detection systems were rated as Top Tier, 2nd through 5th tiers. Significant weightings were given to small size and portability criteria independent of intended function or performance. Due to the nature of the survey and the evaluation criteria, a high score on the survey did not necessarily indicate a useful system in the context of a CAF mission.

The BTB was a hands-on evaluation by typical end-users of selected portable and mobile bioanalysis instruments and as such was more aligned with CAF applications than the GDMS survey. The BTB team of Edgewood scientists and military end-users downselected 11 instruments for hands-on evaluation, 5 nucleic acid-based and 6 antibody-based. A panel of standard analytes was used for both nucleic acid and antibody-based evaluations. Using the evaluation criteria and scenario weighting of the criteria, the systems were ranked 1–5 for nucleic acid or 1–6 for antibody-based. There were differences in the rankings between GDMS and BTB.

The purpose of this scientific report is to provide project managers in the CAF with an outline for making decisions on acquisition of bioidentification systems. The report gives an overview of the process and methods that can be employed to evaluate the candidate systems. It is primarily intended for project managers and science/technical officers of Directorate of Chemical Biological Radiological Nuclear Defence, Special Operations Forces Command, and Canadian Forces Health Services Operational Medicine, and also for other organizations considering procurement. Sections 2 (Methods) and 3 (Results and discussion) are technical. Section 4 (Conclusions and recommendations) contains a summary of the evaluations plus guidance for in-house testing and acquisition.

Newer technologies and more advanced detection systems will arise in the future to supplant the current bioidentification systems but the evaluation processes will remain largely constant. The role of DRDC SMEs is to support the CAF in the procurement processes. A procurement process for BioDIM systems is a difficult undertaking. It must match the appropriate BioDIM technologies to the operational requirements of the end-users. The value of conducting thorough, robust performance assessments of potential systems in a relevant environment by impartial operators is very high. Herein is presented an overview for design and execution of technical assessments.

The report has two messages. The first is that assessments of bioidentification instruments for CAF operations are neither simple nor easy. Careful design is required in the planning phase of the test bed. The challenge of the test bed is to evaluate the underlying system hardware, independent of the reagents, sample preparation and operator skill. The second message is that this challenge is tractable as shown herein.

2 Methods and criteria for assessments

The *CBRTS* 2011 source [2], published by US Army Edgewood Chemical Biological Center (ECBC), covered an assortment of surveillance instruments and systems that had the capability to detect or identify potential chemical/biological/radiological threat agents. The information, technical specifications and performance data concerning the instruments were collected by means of a detailed questionnaire that was prepared by ECBC and provided to the participating companies and vendors. All input data for the survey was vendor-supplied.

A total of about 280 systems were included in the survey. Many of the systems were at a high level of maturity, commercially available or close to. The survey assessed and scored the vendor-supplied information based on a list of questions that addressed four scenarios of operation: 1) man portable/field use, 2) mobile and field laboratory, 3) diagnostic use or point-of-care laboratory, 4) high-sensitivity high-throughput analytical laboratory. The four scenarios had different objectives and requirements for operations. The evaluation criteria, 14 in total, were created and grouped under the four headings of effectiveness, logistics, operations and agents detected as indicated in Figure 1. The relative importance of each criterion to each scenario was assigned a weight shown in Table 1. For each particular scenario, each instrument was scored 0–100 points for each evaluation criterion in Figure 1, based on the imputed performance from the survey data. The final step in the evaluation model was to weight the criteria by scenario according to the assigned weightings in Table 1.

The *GMDS* (2014) [3] source used a similar model, also based on vendor-supplied input. Minor modifications in the 2014 edition were increased focus on chemical and radiological detection technologies, greater number of questions for increased input of information and specifications, revised scoring and ranking system. A total of 304 technologies/systems were evaluated.

In contrast to the above surveys the *Edgewood BTB* (2013) [4] was a hands-on, end-user evaluation of a small number of downselected systems (ca. 11, Table 2) from an initial candidate pool of 30–40 systems. In establishing the candidate pool, some consideration was given to the 2011 survey but most of the selection process was based on a request for information document that was created by ECBC scientists and supplied to the instrumentation industry.

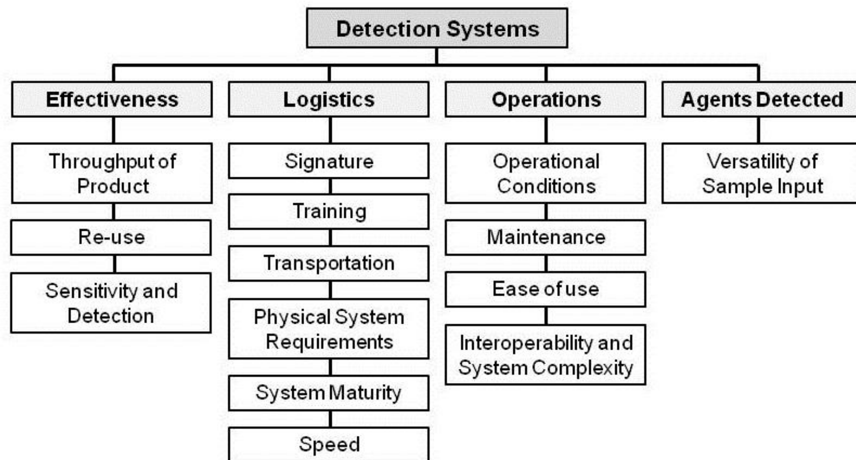

Figure 1: Physical and performance criteria for instrument evaluation (adapted from Reference 1).

Table 1: Distribution of scenario evaluation weightings.[1]*

Evaluation criterion	Field use	Mobile laboratory / Field laboratory	Diagnostic use and point-of-care laboratory	High-sensitivity, high-throughput analytical laboratory
Throughput of product	0.03	0.05	**0.11**	**0.20**
Re-use	0.06	0.03	0.02	0.02
Sensitivity and detection	0.03	**0.12**	**0.24**	**0.27**
Signature	0.06	0.04	0.03	0.01
Training	0.01	0.01	0.01	0.01
Speed	0.06	0.06	0.06	0.06
Transportability	**0.24**	**0.12**	0.01	0.01
Physical systems requirements	**0.16**	**0.12**	0.08	0.03
System maturity	0.01	0.01	0.01	0.01
Operational conditions	**0.14**	0.10	0.05	0.01
Maintenance	0.06	0.06	0.05	0.03
Ease of use	0.07	0.06	0.06	0.05
Interoperability and system complexity	0.06	0.05	0.04	0.03
Versatility of sample input	0.01	**0.17**	**0.23**	**0.26**
Evaluation total scores	1.00	1.00	1.00	1.00

* Adapted from Reference 1.

[1] The weighting represents the relative importance of each criterion to the particular scenario of use. The highly weighed criteria are in bold face.

2.1 Relevance to Canadian Armed Forces

Of the four scenarios of operation, two are more relevant to CAF.

1. Man portable/field use

   Field-use systems would be employed by military or first responders in outdoor or offsite-indoor locations. The systems would be easy to carry, compact (likely handheld) and preferably battery powered, able to operate in heat/cold, high/low humidity. The analysis process would require minimal sample preparation and minimal cleaning between runs. Reagents would likely be pre-loaded in the instrument or in disposable cartridges. Signatures (e.g., mechanical ventilation sound, electromagnetic radiation including thermal radiation, instrument sound and screen light) would be low so not to draw attention to the operators.

2. Mobile laboratory

   The mobile laboratory would be a controlled environment for analytical systems used within. Although electrical power and workspace would be available, small size is still a priority since there would be other analytical devices present. Additional equipment such as mixers and mini-centrifuges would be available for sample preparation. The mobile laboratory would likely operate for extended periods of time and possess some storage space so moderate amounts of consumables would be available for the analysis systems. A larger number of threat agents would be identifiable by the mobile laboratory system than by a man-portable/field-use instrument.

   The evaluations from the CBRTS (2011) [2] for man-portable/handheld and mobile laboratory bio-analysis systems are reproduced in the Appendix 1 (AP.1-1a, b, c). The information and data inputs for the survey were vendor-supplied technical specifications; no actual testing was carried out. The overall rankings were based on the vendor-reported performance. Still there is utility to the survey, namely:

   a. The survey shows that there are many COTS systems available. Out of 282 vendor surveys 60 systems were listed for field and mobile scenarios. However the ratings were not primarily determined by performance or utility, thus it would not be recommended to make a major procurement based on the 2011 survey results.

   b. The authors of the survey have created a model process for evaluation and assessment. The approach presented in Figure 1 and Table 1 could be applied by another party or organization interested in carrying out a survey or carrying out actual assessments of bioidentification systems. The user-specific requirements, criteria and weighting could be modified to suit the other party but overall the survey is a useful starting point for an assessment process leading up to procurement. It is useful to compare the relative weightings in Table 1. Sensitivity, versatility, and throughput are highly weighted for fixed-site scenarios, i.e., diagnostic laboratory and high-throughput laboratory but less weighted for field use and mobile laboratory. Transportability, physicality and operations have increased weights for field use and mobile laboratory. The fact that sensitivity is weighted at 0.03 and 0.12 for field use and mobile laboratory, respectively, (compared to approximately 0.25 for diagnostic and high throughput) does not mean that the actual sensitivity is not important, rather that there are other highly weighted criteria for the non-fixed-site instruments.

c. GDMS (2014) [3] was an updated version of the 2011 survey having a larger list of questions and covering greater number of systems. As with the 2011 survey, it would not be recommended to make major procurement decisions based on the GDMS.

2.2 Biosensor Test Bed

A major source for the present report was the BTB 2013 [4]. The BTB process evaluated the assay performance of a downselected subset of COTS (or near COTS) instruments most of which were contained in the 2011 survey. Downselection was achieved through review of potentially suitable detection/identification instruments by ECBC personnel that included market surveys and correspondence with vendors. Factors included in the downselection processes were assay availability, logistics, and vendor interest and motivation. Overall about 30–40 instruments were assessed and 11 were selected. The list of vendors and technologies is given in Appendix 1. The purpose of the BTB was to provide unbiased technical evaluation by highly skilled technologists/scientists and by actual military end-users. For the two scenarios relevant to the BTB, i.e., mobile laboratory and man portable/field use, the downselected identification systems were grouped by scenario and assay type as shown in Table 2. Two assay types used in the BTB were nucleic acid assay, specifically polymerase chain reaction (PCR), and antibody-based (immunoassay). These assay types are described briefly in Appendix 2.

*Table 2: Classification of identification instruments by scenario and assay type.**

Assay type - technology	Instrument	Scenario	
		Handheld / man portable	Mobile laboratory
Nucleic acid	Film Array by Biofire		√
	Liat by IQuum	√	√
	RAZOR EX by Biofire	√	√
	T-COR4 by Tetracore	√	√
	Genedrive by Epistem	√	√
Antibody	Cartridge Reader MSD	√	√
	NIDS by ANP	√	√
	Spirit by Seattle Sensors		√
	SpinDx by Sandia		√
	MagPix by Luminex		√
	RAPTOR by Res Int'l	√	√

* Adapted from Reference 3.

The two assay types are fundamentally different analytical technologies and can be considered orthogonal. Thus comparison between the two methods for the same agent was not a simple matter. NATO doctrine indicates that during an operation, both orthogonal methods are likely to be deployed [5]. Deploying immunoassay and PCR together would enhance the capability to

make reliable determinations based on the output analysis data. Additionally, the current approach in PCR-based bacterial diagnostics (medical, clinics, hospitals) is to target both plasmid and chromosomal loci which in turn provides an extra level of confirmation within the assay. The presence of multiple copies of plasmids per cell (~3–20) provides a greater number of target molecules per sample than would genomic loci. However due to plasmid mobility and variability there is decreased identification capability based on plasmids alone [6], and inclusion of a plasmid-independent species-specific genetic sequence marker would be advantageous [7]. This approach of multiple target loci in PCR might be useful for mobile laboratory or man-portable systems if the deployed systems could accommodate it.

2.3 Analytes and reagents

Inactivated biothreat agents and antibodies for the test bed were acquired from the Critical Reagent Program (CRP) [8] and are listed in Appendix 3. The technical specifications for the agents are presented in Table 3. Details and explanation of the terms used in the column headings are given in Annex A this report.

*Table 3: Specifications of bacterial and viral agents used in assessments.**

Agent†	Concentration[1] CFU/mL or PFU/mL	Genomic Equivalents[2] (GE)/mL	GE/CFU Ratio	Estimated Genome Size (kbase)[3,4]	GE/ng of nucleic acid[5]
B. anthracis Ames	$6.68 x 10^{8}$	$1.59 x 10^{9}$	2.38	5227	$1.77 x 10^{5}$
Y. pestis CO92	$3.01 x 10^{9}$	$4.17 x 10^{9}$	1.39	4830	$1.92 X 10^{5}$
VAC[6] Elstree (Lister)	$1.31 x 10^{9}$	$1.89 x 10^{9}$	1.44	189	$4.90 x 10^{6}$
VEE[7] virus, TC-83	$1.00 x 10^{10}$	$1 x 10^{10}$	1.00	11.4	$1.62 x 10^{8}$

* Adapted from Reference 2. See Annex A for explanation of column headings.

† See Appendix 3.

[1] Colony forming units (CFU/mL) for bacteria or plaque forming units (PFU/mL) for virus.

[2] Data from CRP Certificate of Analysis. Genomic equivalents for the VEE antigen were not reported on the Certificate of Analysis. For this study a ratio of 1.00 was used.

[3] Genomes Online: www.genomesonline.org. Accession Numbers Gc00136 and Gc00064 for *B. anthracis* and *Y. pestis*, respectively.

[4] GeneBank. Accession Numbers DQ121394.1 and L01443.1 for VAC and VEE, respectively.

[5] Endmemo Online: http://www.endmemo.com/bio/dnacopynum.php.

[6] Vaccinia *variola* Elstree (Lister) strain.

[7] Venezuelan equine encephalitis virus.

The agents were selected to represent the classes of threat agents: bacteria (Gram-positive, Gram-negative), virus and protein toxin. The concentrations of CRP bacterial and viral agents were provided in colony forming units (CFUs) and plaque forming units (PFUs), respectively. The CFUs and PFUs had been previously determined in the Critical Reagents Program by

standard microbiology culture methods, i.e., growing the microbes in the laboratory. After the growth assays (CFU/PFU determinations), the bacterial and viral agents were inactivated by gamma irradiation for inclusion in the CRP inventory and for subsequent use in assay development and in testing. The growth data only indicated original concentration of viable microbes in the culture-preparation and did not account for microbes that would not grow or were dead, or for lysed microbe debris. Hence the stock preparations of each agent used in the BTB could have contained significant amounts of material that would be "unaccounted for" in the growth assays but might nonetheless provide signal in the test bed assays. Thus the actual or effective concentration of detectable materials for the nucleic acid or antibody-based assay could have been significantly greater than the growth data would indicate. In other words, there was uncertainty in actual concentration of analyte. Immunoassay measures the amount of a signature target analyte (often a protein or carbohydrate component of the microbe coating) present in the sample. According to the reagent specifications (see Appendix 3) each BTB immunoassay used at least one monoclonal antibody reagent. So the analyte detected by the assay would have been a specific molecular component of the microbe. This component would be present in large number of copies per microbe (i.e., 1 microbe would possibly yield ~10^2–10^5 analyte molecules). The target for a PCR assay was a genomic DNA sequence indicative of the microbe and usually present as one target sequence per microbe (or at most a few copies per microbe).

2.4 Instruments

Details on the instruments and systems used in the assessments are given the BTB [4]. Vendor details are given in Appendix 1 (Table AP.1-1) of this report. Product information is available on the vendor websites.

The instruments were used according to the vendor manuals. In the BTB the vendor-provided limit of detection (LOD) was the starting concentration for test assays of the respective instrument. If the instrument was able to obtain triplicate positive readings at the vendor-provided LOD concentration, then this value was taken to be the BTB-determined LOD. If not, the test assay was repeated with 10-fold increase in concentration until triplicate positive readings were obtained. The concentration that yielded triplicate positive readings was taken as the BTB-LOD.

3 Results and discussion

3.1 Preliminary laboratory assessment of identification systems

A preliminary laboratory assessment of the downselected set of instruments was carried out by ECBC personnel to verify the ability of systems to detect the panel of analytes. For instruments that did not contain manufacturer-ready assays, ECBC provided support for assay development including antibody reagents obtained through the Critical Reagent Program (CRP). Following the BTB laboratory assessment, the instruments were evaluated for suitability as mobile laboratory and man-portable/field-use applications, based on a list of attributes shown in the upper portion of Table 4. For the man-portable applications an additional set of specific attributes was included and shown the lower portion of Table 4. The attributes pertained to basic properties such as size (instrument really was portable and handheld) and battery (sufficient energy available to do a useful amount of analysis when unplugged) and were graded as pass/fail.

*Table 4: Preliminary assessment criteria for bioidentification instruments.**

List of attributes	Mobile laboratory	Man portable handheld
Ease of use	√	√
Ease of viewing data	√	√
Ease of interpretation of data	√	√
Supporting documentation	√	√
Training simplicity	√	√
Safety	√	√
Cleaning and maintenance	√	√
Specific suitability factor for man-portable/field-use instruments		
Size		√ √
Battery power		√ √
Sample preparation requirements		√ √
Reagent stability		√ √
End-user requirements		√ √

* Adapted from Reference 3.

√ = scored criteria based on performance during laboratory assessments.

√ √ = additional assessment of overall suitability of instruments for field use (handheld).

Man-portable handheld criteria were evaluated as pass/fail.

3.2 Limits of detection

The lower limits of detection for the selected instruments were determined for the panel of analytes (see Table 3) and are shown in Table 5. For botulinum neurotoxin serotype A (BoNT/A) 4 of 6 antibody-based instruments were able to report an LOD. For nucleic acid technologies only Epistem Genedrive was able to report a LOD for BoNT/A. The Genedrive instrument was successful because its assay chemistry had been modified to detect enzyme activity of the toxin using a fluorescently labeled substrate.

*Table 5: Limits of detection of identification instruments.**

Technology	Instrument	Analyte LOD (singleplex/multiplex)				
		BA CFU[1]/mL	YP CFU[1]/mL	VAC PFU[2]/mL	VEE PFU[2]/mL	BoNT/A ng/mL
Nucleic acid (PCR)	Film Array by Biofire	$5x10^3$ / $5x10^3$	$5x10^1$ / $5x10^2$	$1x10^3$ / $1x10^3$	$1x10^6$ / $1x10^6$	NA / NA
	Liat by IQuum	$1x10^3$ / $1x10^3$	5 / 10	$2.5x10^3$ / $2.5x10^3$	$2x10^3$ / $2x10^3$	NA / NA
	RAZOR EX by Biofire	$1.3x10^4$ / $1.3x10^4$	$1.3x10^3$ / $1.3x10^3$	NA / NA	NA / NA	NA / NA
	T-COR4 by Tetracore	$3.2x10^4$ / NA	$1.3x10^1$ / NA	NA / NA	NA / NA	NA / NA
	Genedrive by Epistem	$1.9x10^4$ / NA	$2x10^2$ / NA	$3.4x10^4$ / NA	NA / NA	$1x10^4$ / NA
Antibody-based (Immuno assay)	Cartridge Reader MSD	$1x10^5$ / $1x10^5$	$1x10^5$ / $1x10^5$	$1x10^7$ / $>1x10^8$	$1x10^8$ / $1x10^8$	$1x10^2$ / $1x10^3$
	NIDS by ANP	$1x10^7$ / $1x10^7$	$1x10^6$ / $1x10^6$	$>1x10^8$ / $>1x10^8$	$1x10^9$ / $1x10^9$	$5x10^1$ / $5x10^2$
	Spirit by Seattle Sensors	$>1x10^7$ / NA	$1x10^7$ / NA	$1x10^8$ / NA	$1x10^8$ / NA	$1x10^3$ / NA
	SpinDx by Sandia	NA / NA	NA / NA	NA / NA	NA / NA	NA / NA
	MagPix by Luminex	$1x10^5$ / $1x10^7$	$1x10^5$ / $1x10^6$	$1x10^7$ / $1x10^8$	$1x10^8$ / $1x10^8$	$1x10^3$ / $1x10^3$
	RAPTOR by Res Int'l	$>1x10^7$ / $>5x10^6$	$5x10^7$ / $5x10^7$	$>1x10^7$ /> $1x10^7$	NA / NA	NA / NA

*Adapted from Reference 4.

BA = inactivated *Bacillus anthracis* (Gram-positive spore-forming bacillus), YP = inactivated *Yersinia pestis* (Gram-negative bacterium), VAC = vaccinia *variola*, VEE = Venezuelan equine encephalitis virus, BoNT/A = *Clostridium botulinum* neurotoxin serotype A (protein).

[1] CFU = colony forming unit.

[2] PFU = plaque forming unit.

Total volume per assay was 0.25 mL. The LOD by total amount of analyte is determined from the above concentration multiplied by 0.25 mL. NA = not applicable.

For nucleic acid analysis, the LODs were 10^1–10^4 CFU/mL for bacteria and 10^3–10^6 PFU/mL for viruses (Table 5). All systems evaluated were able to detect *Bacillus anthracis* and *Yersinia pestis*. For the virus assays two of the systems did not have the requisite PCR reagents integrated into the assay kits so test results were unavailable. Biofire FilmArray was the only nucleic acid system tested that had more than one target DNA locus per agent; it had the ability to amplify chromosomal and plasmid sequences simultaneously. The tests also showed that toxins in general constitute a challenge for nucleic acid amplification methods since toxin molecules are rarely composed of nucleic acid. Residual DNA, if present in sufficient quantity, might provide a signature of the toxin-producing organism. However three nucleic acid instruments tested were unable to detect the presence of residual DNA in the reference analyte material, which was highly purified reagent-grade botulinum toxin A. Highly purified reagent-grade botulinum toxin is difficult to produce or obtain even in small amounts. Large-scale amounts of purified toxin are even more difficult to obtain. In a malicious bioincident, lesser-purified preparations of toxins would likely be used and would likely contain detectable amounts of residual nucleic acids. Detection of DNA signatures of C*lostridium botulinum* in unpurified or semi-purified samples of BoNT has been demonstrated previously [9], [10]. The modified version of the Epistem Genedrive system demonstrated a LOD for BoNT/A of 1×10^4 ng/mL. For antibody-based analysis LODs were 10^5–10^7 CFU/mL for bacteria and 10^7–10^9 PFU/mL for viruses. All systems tested were able to detect *Bacillus anthracis* and *Yersinia pestis* bacteria.

The LODs for microbe analytes would be affected by the method of inactivation. In the BTB analytes were inactivated by gamma irradiation. Other means of inactivation, such as heat, formaldehyde, chlorine, etc., could lead to different LOD results.

3.3 Mobile laboratory assessment

The mobile laboratory assessments were conducted by typical operators from the US Army 20th Support Command in the Heavy Mobile Expeditionary Laboratory located at Aberdeen Proving Ground, MD. Sample preparation was minimized. For nucleic acid analysis, inactivated samples were used "as-is" for instruments that possessed integrated sample preparation. Otherwise samples were treated with Qiagen DNeasy (a commercial product, see Appendix 3) to extract and purify the nucleic acid analytes. For antibody-based instruments, samples of inactivated analytes in buffer solution were presented without any sample preparation. Assessments were made based on seven attributes listed in the upper portion of Table 4. The scores and ranking are shown in Table 6.

*Table 6: Scores and rankings mobile laboratory instruments.**

Technology	System	Overall Score	Ranking	TRL[1]	Usability[2]	Spec[3]
Nucleic acid	Film Array by Biofire	100	1	7	5	p. 28
	Liat by IQuum	100	1	7	5	34
	RAZOR EX by Biofire	98	3	7	5	30
	T-COR4 by Tetracore	97	4	7	5	36
	Genedrive by Epistem	87	5	5	5	32
Antibody	Cartridge Reader MSD	100	1	6	5	42
	NIDS by ANP	97	2	7	5	38
	Spirit by Seattle Sensors	82	3	5	4	48
	SpinDx by Sandia	80	4	4	5	46
	MagPix by Luminex	78	5	6	3	40
	RAPTOR by Research Int'l	68	6	6	2	44

* Adapted from Reference 4.

[1] Technology Readiness Level is 1 to 9, see Appendix 4.

[2] Usability score is 1 to 5.

[3] Technical specifications from BTB (Reference 4, pages as indicated in Table 6).

3.4 Field assessment for man-portable/field-use instruments

The field assessments were designed to determine the ability of the candidate instruments to detect the panel of analytes in a non-laboratory field setting. The scoring was similar to that of the mobile laboratory. The assessments were performed by members of the 56th Chemical Reconnaissance Detachment 5th Special Forces Group and US Army 22nd Chemical Battalion at Skippers Point Training Area, Aberdeen Proving Ground. Sample preparation for nucleic acid analysis and antibody-based analysis was minimized in the same manner as the mobile laboratory assessments. The scores and rankings for man-portable/field-use instruments are shown in Table 7. The field performances and rankings of the man-portable/field-use instruments were similar to that in the mobile laboratory assessment. This suggests that there could be some commonality of hardware for CAF procurement. If CAF were to undertake assessments of biodetection instruments, the above processes are highly useful as a starting point.

*Table 7: Score and ranking man-portable/field-use instruments.**

Technology	System	Overall Score	Ranking	TRL[1]	Usability[2]	Spec[3]
Nucleic acid	Liat by IQuum	96	1	7	4	p. 34
	T-COR4 by Tetracore	96	1	7	5	36
	RAZOR EX by Biofire	86	3	7	4	30
	Genedrive by Epistem	79	4	5	3	32
Antibody-based	Cartridge Reader by MSD	99	1	6	5	42
	NIDS by ANP	95	2	7	5	38
	RAPTOR by Research Int'l	82	3	6	4	40

* Adapted from Reference 4.

[1] Technology Readiness Level is 1 to 9, see Appendix 4.

[2] Usability score is 1 to 5.

[3] Technical specifications from BTB (Reference 4, pages as indicated in Table 7).

3.5 Discussion of Biosensor Test Bed assessments: drilling-down

The BTB was undertaken to evaluate the performance of the downselected set of identification systems so that comparisons could be made with respect to suitability for operation. In order to draw insights and conclusions from the BTB report [4], it is useful to closely analyse what was actually measured and how. The analytical approach provided in this section is useful for future CAF-sponsored assessments. Having the analytical tools to "drill-down" into test bed data enhances the decision-making process for procurement.

Table 8 gives the mean LODs by assay method for the BTB analytes. In terms of microbiology culture data (CFU or PFU), both nucleic acid assays and antibody-based assays yielded lower mean LODs (estimated number of microbes) for bacteria than for viruses. Furthermore, nucleic acid assays yielded lower LODs than antibody-based assays. These data in Table 8 are noteworthy of a general trend but should be treated with "caution" because it was not possible to determine LODs in terms of actual concentration of analyte molecules based on culture data alone. However some rough comparison of PCR to immunoassay is possible. Published reports of FilmArray assays concur with the data given in Table 5 whereby LODs of 100–1000 genomic copies are attainable [11]. Thus in the BTB on a molecular basis, PCR was able to resolve fewer molecules (i.e., 10^2–10^3) than the most sensitive immunoassay (10^4–10^5 molecules). The LODs for nucleic acid and antibody-based assays are discussed in greater depth in Annex A of the report.

The data in Table 8 indicate that the variation of LOD from instrument-to-instrument for a particular analyte and assay method (e.g., analyte BA, nucleic acid assay) was about 1 log unit. Although 1 log unit variation (a factor of 10) might seem large in many circumstances, it is not uncommon in reporting of LODs and it is consistent with the methods used, namely serial 10-fold dilution of analyte. For nucleic acid assays of YP the variation of LODs was also about 1 log unit,

but the mean LOD for YP was 2 log units less than BA. Inspection of the ordered LODs (lowest to highest) for nucleic acid assay of BA and YP (Table 5) shows that the orders were not the same, which would suggest that the determined values for LOD are not exclusively hardware (i.e., instrument) dependent. Differences in orders of LODs are also seen for nucleic acid assays of virus (VAC versus VEE) and for antibody-based assays, again showing that LOD is not exclusively hardware dependent.

To restate the problem: Meaningful weight- or molecule-based LODs in a test bed can be difficult to obtain. If the material to be assayed is a purified molecule such as BoNT (protein) of a known molecular weight or a nucleic acid molecule of known sequence, then a meaningful LOD based on actual number of analyte molecules (or weight) is possible. Thus for BoNT the LOD of the instrument can be determined by serial dilution of the analyte standard. From these data the total number of analyte molecules required for a positive signal can be calculated [12], [13]. For microbes such as those shown in Table 8, concentrations based on growth data (CFU, PFU) could yield LODs that vary greatly. The CFU and PFU only indicate what grew, not the amount of material in the sample that generated the signal. For test bed assays, it is advisable to characterize the microbe samples by cell counting and/or simple and common protein and nucleic acid quantitation (see Annex A for further discussion).

For the goal of expedient evaluation of instrument hardware, test samples composed of standard purified proteins and purified PCR products (nucleic acids) are a useful complement to the inactivated microbe samples such as in Table 5. The purified single molecular analytes will better demonstrate the underlying instrument performance. Assays of inactivated microbe samples will produce knowledge about the effectiveness of the sample preparation, the robustness of the reagents and assay methods, plus the skill of the operators. The ability of the test bed to separate these issues is critical. For procurement, CAF is buying hardware, but not necessarily sample preparation, reagents or operator skills.

Table 8: Mean limits of detection for identification instruments.

Technology		BA CFU[1]/mL	YP CFU[1]/mL	Mean bacteria	VAC PFU[2]/mL	VEE PFU[2]/mL	Mean virus	BoNT/A † ng/mL
Nucleic Acid*	Mean conc. Mean log (SD)	$8x10^3$ 3.9 (0.6)	1×10^2 1.7 (0.9)	1×10^3 2.8	$2x10^3$ 3.6 (0.8)	$5x10^4$ 4.7 (n/a)	$1x10^4$ 4.0	$1x10^4$ 4.0
Antibody-based*	Mean conc. Mean log (SD)	$4x10^6$ 6.2 (1.1)	$1.3x10^6$ 6.0 (1.0)	$2x10^6$ 6.2	$3x10^7$ 7.5 (0.6)	$2x10^8$ 8.3 (0.5)	$6x10^7$ 7.8	$4x10^2$ 2.4 (0.7)

* Includes systems listed in Table 5. The mean data above were derived from the mean of LODs given in Table 5. SD = standard deviation.

†Molecular weight of BoNT/A is 150 kDa [13].

BA = inactivated *Bacillus anthracis* (Gram-positive spore-forming bacillus), YP = inactivated *Yersinia pestis* (Gram-negative bacterium), VAC = vaccinia *variola*, VEE = Venezuelan equine encephalitis virus, BoNT/A = *Clostridium botulinum* neurotoxin serotype A (protein).

[1] CFU = colony forming unit.

[2] PFU = plaque forming unit. Total volume per assay was 0.25 mL.

3.6 Factors for developing an effective test and evaluation strategy

3.6.1 Goals and objectives

For undertaking a test bed study, it is useful to consider project management theory [14] for defining the goals and objectives. The goals are high-level statements that provide the overall context of what the test bed study is attempting to achieve, namely, recommendations to procurement managers based on unbiased assessments of biodetection/identification instruments. The objectives are lower level statements that describe specific products, deliverables or in this case work units. Examples of objectives would include:

- define assessment criteria and provide relevant weighting, e.g., Figure 1 and Table 1;
- define roles and scenarios for use of instruments, e.g., mobile laboratory and man portable;
- define common analytes and reagents, e.g., Appendix 3;
- determine LODs of test standards on COTS and prototype systems, e.g., Table 5; and
- score and rate systems, e.g., Tables 6 and 7.

3.6.2 Critical elements to incorporate into a test bed

3.6.2.1 Assay design

If the intention of the test bed is to assess a variety of instruments, a single common assay design might not be possible especially when the instruments are based on different technologies (PCR, immunoassay, toxin activity). However defining and maintaining common methodologies within a technology (e.g., immunoassay) are feasible and also beneficial for a fair and transparent evaluation. Assay design will take into account the openness and the architecture of each instrument, that is, the ease or difficulty in changing analytes and reagents or in modifying sample preparation routines.

3.6.2.2 Target choice

For initial evaluations, proxy assays that employ reagent-grade specific molecules as analytes provide good standards. Also proxy assays can be used to establish an expectation value for the more complex analytes. When using real-world complex samples or challenge materials, such as inactivated agents or nonpathogenic microbe strains, it is important to take into consideration what is being measured. So for PCR assays, is the target a genomic or plasmid locus? For immunoassay, is the analyte a specific known molecule (protein, polysaccharide, etc.) or unknown component of the complex sample?

3.6.2.3 Cross platform comparisons

Due to inherent differences in instrument architecture, reagent constraints, and technology readiness level, direct comparisons for assay LODs can be difficult. Where possible commercially

available analyte samples should incorporated into in the test bed. The use of such samples, which are usually purified and characterized proteins of known molecular weight or purified nucleic acids of known sequence, focuses the assessment onto the system hardware and away from reagent design, sample preparation or operator skill.

3.6.2.4 Study limitations

The BTB study focused on the criterion of sensitivity by determining the LOD of the instruments for the test analytes. Another criterion in bioidentification is the selectivity of the assay, that is, the ability to detect the analyte in a complex medium or matrix and to discern near-neighbour organisms from the organism of interest. However selectivity was not directly addressed in the BTB. In general selectivity is more a problem of assay design, sample preparation and reagents (often referred to as wetware) than of the instrument hardware. Still selectivity is indirectly related to the hardware by way of the flexibility and openness of the instrument architecture, i.e., how readily assay design, sample preparation or reagents can be modified to remedy problems. A limitation of the Edgewood BTB was that little was learned about selectivity using the panel of threat agents. In a test bed, selectivity becomes a separate problem and requires special attention to near neighbour targets and the sample matrix. Further discussion on selectivity is given in Annex A. Overall the limitation of the test bed study is inability to completely separate the performance of instrument hardware from that of sample preparation, reagents and operators. For sponsors, recognition and mindfulness of test bed limitations will lead to better test bed design.

4 Conclusions and recommendations

Most of the instruments evaluated in the *Edgewood BTB* were available as COTS products or high-functioning demonstrator models during the test period. Many of the instruments have had subsequent development since the BTB was undertaken. The BTB report recognized the underlying problems with assay design and direct comparison of results. Some of the analysis systems included on-board sample preparation, others did not. For nucleic acid analysis, sample preparation was limited to an easy-to-use commercial product (Qiagen DNeasy, see Appendix 3). The test results, such as LODs, were compiled based on the concentration of sample presented to the instrument. The volume of sample used in each test was also reported in Table 5, thus the total amount of sample presented in each test was easily obtainable (multiplying concentration by volume). LODs were given in CFU for bacteria and PFU for virus. The LODs reported by the BTB operators were often higher by a factor of 10 than LODs supplied by the vendors. This finding is not surprising or unreasonable given differences in reagents, sample preparations, assay protocol and operators.

The BTB operators gave high ratings to the non-COTS Meso Scale Discovery (MSD) Cartridge Reader. The instrument was antibody-based and delivered high sensitivity, electrochemi-luminescent detection in about 30 minutes. Since the BTB period, the company now offers commercial versions of the Cartridge Reader technology. For PCR-based analysis Iquum Liat™ (Lab-in-a-Tube) and Biofire Diagnostics FilmArray were rated highly. The Liat system is now available through Roche Diagnostics. The FilmArray PCR system integrates sample preparation, amplification, detection and read-out display as a single operation. A pouch containing all sample preparation and reagent materials provides exceptional ease of use. The FilmArray platform was capable of analyzing up to 12 samples simultaneously, including 3 targets of *B. anthracis*, 2 targets of *Y. pestis*, 2 targets of VEE virus and 2 targets of orthopoxvirus. The Tetracore instrument was also highly rated, only slightly below Liat and FilmArray, for field-deployable PCR. Since the Test Bed period, Tetracore has redesigned the system for processing eight samples simultaneously at the low end of the ratings were Sandia SpinDx, Seattle Systems SPIRIT and Research International RAPTOR. The antibody-based Luminex MAGPIX had assay flexibility, multiplex capability and high throughput. In size it was deemed too large for field use (ca. 18 kg plus notepad computer) but scored well as a reliable mobile laboratory instrument.

Even with well-designed test and evaluation protocols, well characterized analytes (inactivated biothreat agents), and well trained operators, meaningful comparison of LODs with respect to analytical method or bacteria-vs-virus is not readily achievable on a quantitative basis, especially when sample concentrations are given in CFU or PFU.

A conclusion that can be drawn from the review and analysis of the BTB is that a direct singular answer to the question of “which instrument is better?” is not likely to be attained. The problem is complicated in that there are many dimensions for comparison. The final test scores are based on a composite of factors including origin of sample, origin of reagents, sample preparation, assay methods, etc. It is also important understand the nature of the analytes that are being used. The Edgewood BTB employed real (but inactivated) biothreat agents shown in Table 3. Even to knowledgeable operators there is a problem making sense of the specifications of the bacterial

and viral agents in Table 3 and understanding how these specifications (genome equivalents (GE), genome size and GE/CFU ratio) affect the test results. Annex A to this report gives an explanation of the specifications and how they apply to the test bed.

4.1 Main conclusion

The main conclusion of this report is that the problem of performance assessment of bioidentification instruments is difficult but tractable. If we dissect the problem and apply what we know to it, then the problem area will shrink to a manageable size. The outcome of a test bed will be a selection and procurement process that delivers a useful bioidentification systems to CAF based on tractable challenge materials, methods and LOD criteria.

4.2 Main recommendation

The main recommendation to CAF that underlies any test bed assessment process is that CAF should rank the performance and logistics requirements of the desired bioidentification system prior to undertaking a test bed. The requirements should be based on the scenarios of operation. Figure 1 and Table 1 in this report give the criteria and weightings used in the BTB. A simpler version of the criteria scheme would suffice for CAF, one that gives a short list of essentials. The second part of this recommendation is that CAF would ask for help from SMEs and end-users.

4.3 Further recommendations

4.3.1 Assessment of instruments (hardware)

A caveat given in the Edgewood BTB and reiterated here for CAF is that the performance of an identification instrument in a standardized test bed assay is not a true evaluation of the underlying technology. A recommendation is, where possible, that the performance of the underlying technology and the performance of the assay should be evaluated separately and independently. For example, in immunoassays, if the antibody and target analyte do not bind strongly then the overall performance will suffer even though the instrument hardware performed well. Also the sample preparation procedure and assay dilutions will affect assay performance, independently of the instrument. For PCR assays, good reagents and good primer design are required to obtain satisfactory results, again independent of the hardware/software of the instrument.

4.3.2 Assessment criteria

A recommendation for CAF procurement managers is that the sensitivities or LODs of various instruments should not be considered as the overriding criterion. The data in Tables 5 and 8 indicate that the better performing systems have LODs in similar ranges, i.e., within a log unit (+/-) for a given analyte and assay method. Instruments that have LODs more than a log unit outside the range (i.e., 2 logs above the mean) might be considered deficient in the LOD criterion. This recommendation does not discount LOD or sensitivity; they are important attributes of an instrument. However a test bed evaluation process should not be a contest of instrument LODs. The convenience of operation and reliability of the deployed systems are important criteria

although establishing metrics may not be simple. Even in the absence of metrics, qualitative evaluations for such are achievable. Several of the systems detailed in the BTB report and reviewed here are possible candidates for acquisition.

4.3.3 Decision feedback

CAF managers are recommended to engage DRDC SMEs at all levels of the assessment and selection processes. A strong feedback loop allows the SMEs to better understand the CAF decision-making processes. This in turn leads to better support to CAF from DRDC and better decisions.

4.3.4 Reagents and analytes

For simple initial evaluation of instruments or systems, it is recommended that assessment assays be designed to use reagents that are commercially available (supplied with detailed specifications sheets) and require little-to-no development. Reagents of this type are less prone to operator variation or artifact. These proxy assays might not represent a threat agent analyte (as would inactivated *Bacillus anthracis*) but for the most part the performance of the instrument is independent of the specific analyte material being tested. For example in receptor-ligand binding (immunoassay), the signal obtained in a proxy assay is likely to be close to the maximum for the hardware system. The assay serves a convenient range finder for more realistic analytes. As well, if something goes awry in the assay process, the operator can quickly return to the proxy for troubleshooting. For PCR assays numerous primer-target sets are available from molecular biology supply companies for evaluation of performance. Instrument performance is likely to be close to maximum with characterized commercial primer-target reagents.

4.4 The challenge

There is no likely ideal candidate technology that will be suitable for all BioDIM requirements. Nonetheless deployable systems for mobile laboratory and man-portable/field-use BioDIM are achievable. Although BioDIM itself (i.e., the process of obtaining results from the deployed analytical systems) might not be the sole factor in the decision-making process for a response to a biological event, it will play an important role. A thorough knowledge of the underlying system technologies plus a thorough understanding of the analysis process is critical to successful operations in the field. DRDC will provide guidance and support to CAF in all stages leading to procurement and deployment.

Most of the systems covered by the BTB report were relatively easy to use at the level of medical technologist or first responder. In the BTB evaluations, the assays were designed to produce readily interpretable outputs. That is, take a sample of a given concentration; do sample preparation if required—then run the assay; compare results (signal output) to blank or baseline; examine the results to determine whether the signal is high enough above the baseline to call positive. In deployment, the systems would have similar ease of use as in the BTB but the samples would be varied and likely contain contaminants and interferents. The samples would be multifacetedly unknown. That is, the sample may or may not contain the pre-selected known analytes, and may or may not contain non-analyte materials of known or unknown variety. There

is no available “app” to definitively tell positive from negative and true from false. The success of field BioDIM will rely strongly on the operators and subject matter experts within the support network. The solutions to BioDIM will be found by formulating the problem as an operator issue rather than a hardware issue. Good hardware is important to the solution; good operators and a strong support network, such as DRDC, are essential.

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Annex A Dimensions and units used for a test bed evaluation

This Annex is an aid to understanding concentrations and units given in Table 3 of the present report and in the *Edgewood Biosensors Test Bed* [A.1]. For test bed evaluations, the measurements should be reported using the International Systems of Units (SI) where possible. The SI consists of seven base units upon which all physical measurements can be made. The system also includes prefixes to denote decade fractions or multiples of the unit names and symbols. For test bed measurements, as discussed in this report, three base units are required: length, mass and amount of substance; the units are metre (m), gram (g) and mole (mol), respectively. In wet chemistry and biology the most often reported measurement of volume is litre (L). Although strictly it is a non-SI unit, it is acceptable for use with SI. A litre is 1/1000 part of a cubic metre. Common fractional units of litre are milliliter (0.001 L = 1 mL = 1 cubic centimeter, cm^3) and microlitre (0.001 mL = 1 μL).

In a test bed the dimensions associated with sample volume are easy to contextualize. A sample from a collector might be several millilitres (a teaspoon amount is 5 mL). For most laboratory work, e.g., test bed analyses, sample solutions are transferred using Eppendorf-style pipettes which can routinely deliver volumes as low as 1 μL. PCR-based analytical systems often require sample volumes of 1–10 μL. Immunoassay systems require sample volumes about 100 μL. For the test bed results described in this report the amount of material being analyzed was expressed in a number of ways that might be confusing and difficult to contextualize, e.g., Table 3 viz., colony forming unit (CFU) for bacteria, plaque forming unit (PFU) for virus, nanograms (ng) for protein toxin, genome equivalents (GE), and genome size (kilobase pairs, kbp). Of these only nanograms is based on SI units.

Microbe size: Bacteria vary in size but are typically of the order of a micrometer, e.g., a bacillus rod is approximately 2 μm length (l) and 0.8 μm in diameter (r = 0.4 μm) [A.2]. The volume of the rod ($V = \pi \cdot r^2 \cdot l$) is about 1.0 μm^3.

For clarity, $1\ \mu m = 1 \times 10^{-4}$ cm,
thus $1\ \mu m^3 = 1 \times 10^{-12}\ cm^3$.

The density of a cell is about 1.05 g/cm^3 [A.3], thus the wet weight of a bacteria cell is about 1.05 pg (i.e., approximately 1 pg). Note: the approximate volume (1 μm^3) and weight (1 pg) of a bacteria cell are useful information to remember. Virus particles also vary in size and are about 1/10 scale of a bacteria, typically about 0.1 μm in length. Thus the volume of a virus particle is about 1/1000 of a bacterial cell, 10^{-15}–10^{-16} cm^3. Virus particles have lower water content than bacteria cells and hence the density is greater, about 1.3–1.5 g/cm^3 [A.3] for viruses without lipid membrane and about 1.2 g/cm^3 for virus with lipid membrane [A.4].

Colony Forming Units: In microbiology a CFU is a measure of the number of viable microbes (bacteria, fungi, etc.) in a sample. Viable means the ability to grow (replicate) under specific laboratory conditions, usually on agar-containing Petri dishes until colonies appear. In principle a single viable cell can replicate and develop into a colony. However cells are prone to stick together, thus the colony seed might be multiple cells. For this reason colony counting may under estimate the number of viable cells in the sample. The CFU count only reflects the concentration

of viable cells in the starter sample. It does not take into account intact cells that do not replicate, broken or dead cells, or non-cellular biological material (soluble nucleic acids, protein, lipids, etc.). A count of the resulting colonies combined with knowledge of the volume of sample spread onto the Petri dish and the sample dilution factor allows the concentration of colony forming units (CFUs) in the original sample to be determined. The concentration of the viable cells in the original sample is expressed as CFU/mL.

Plaque Forming Units: PFU is a measure of the number of infectious virus particles present in a sample. Viruses are not capable of replicating on their own. Plaque assays are usually carried out in agar Petri dishes containing a monolayer of host cells. The host-cell monolayer is infected at varying dilutions of the virus. The infecting virus will replicate within the cell and cause the cell to lyse, thereby infecting the neighbouring cells. In time the infected region (the plaque) is observable by eye or by microscope. A count of the resulting plaques combined with knowledge of the volume of sample spread onto the Petri dish and the sample dilution factor allows the concentration of plaque forming units (PFUs) to be determined. The concentration of the infective virus in the original sample is expressed as PFU/mL.

Genome size: Table 3 of this report gives a literature value for the genome size of *Bacillus anthracis* (BA) as 5227 kbp, determined by genetic sequencing. The genome size is a measure of the amount of DNA in the cell. We can also estimate the amount of DNA in the cell using the size and weight of the notional bacillus cell (2 μm length x 0.8 μm dia.) plus the density of the cell and the data in Table A.1 (DNA content of a cell = 1%). Mass of DNA per cell is 1.05×10^{-14} g or 0.0105 pg. This value is total DNA, i.e., genomic DNA plus plasmid DNA. Plasmids vary in size and in number, typically amounting to 5–25% of total DNA [A.5]. BA possesses 3 plasmids each of variable copy number which combined make up about 25% of the total DNA [A.6], [A.7].

Thus the amount of genomic DNA in BA would be 0.0105 pg x 0.75 = 0.0079 pg = 7.9×10^{-3} pg.

The mass of 1 base pair in a DNA polymer is 615 Da. This can be expressed as

1 mole of base pairs (6.02×10^{23} molecules) has mass of 615 g.

Rearrangement of this relationship gives

1 pg of DNA = 926 Mbase pairs = 0.926×10^9 bp.

Note: remember that 1 pg DNA = 10^9 bp (approximately).

Thus the genomic DNA content of BA can be expressed as

$$(7.9 \times 10^{-3}\ \text{pg}) \times (0.926 \times 10^9\ \text{bp/pg}) = 7.3 \times 10^6\ \text{base pairs.}$$

This estimated value of 7.3×10^6 bp is close to the literature value of 5.2×10^6 bp (see Table 3). The purpose of this exercise was not to check the literature value but to put the literature value into context. The exercise shows that a useful estimate can be obtained from simple arithmetic and basic estimates of mass and size and then applied to analyzing test bed results.

Genomic equivalents: The definition of genomic equivalent (GE) *"is the amount of DNA required in a purified sample to guarantee that all genes will be present. This number increases with the total genome size of an organism and can be calculated by converting the size of a genome in*

base pairs to micrograms of DNA" [A.8]. Basically one intact cell contains 1 GE. The BTB [A.1] makes reference to the certificate of analysis of the Critical Reagents Program for GEs given in Table 3. Although the actual method used to determine GE was not indicated in the BTB, there are several standard methods to determine the GE equivalents for a microbiology culture sample. Cells can be counted using a haemocytometer under the microscope, a Coulter counter, or a flow cytometer. A simple estimate of GE is to determine the cell concentration by performing an optical density measurement. The basic rule-of-thumb is a cell suspension of 1 OD unit (at 600 nm) contains 10^9 cells/mL [A.9]. This approximation is cited often in the microbiology and molecular biology literature. Other methods to determine GE by way of cell content include 1) a standard protein assay that would give the total protein concentration which could be converted to cell concentration (i.e., GE) using Table A.1 and the above estimated cell weight; 2) a standard DNA assay would give total DNA concentration which could be likewise converted to cell concentration (GE) by Table A.1 and the estimated cell weight; 3) GE can be calculated from the measured total DNA concentration and the genome size data shown in Table 3, i.e.,

$$\text{GE} = (\text{Total DNA} - \text{Plasmid DNA}) \div (\text{genome size}).$$

In these ways GE can be determined independently of CFU. Each method would likely give a different estimated value for GE, but there should be some consistency among the estimates. Variations by factors of 2–4 would not be unexpected; variations of 10 or greater would be suspect.

GE/CFU ratio: The ratio of genomic equivalents to colony forming units (GE/CFU) is an indication of the viability and the purity of the microbe sample after it was cultured and harvested. Determination of this ratio requires two independent measurements: the genome equivalents calculated by one (or more) of the methods given above and the CFU obtained from growth culture data. If one cell gives one colony and there is nothing else present in the original culture (just intact viable cells), then the ratio should be equal to 1. In practice because cells clump, the CFU underestimates the number of viable cells. Furthermore a completely clean cell preparation is highly unlikely. Thus the ratio is expected to greater than 1. This is shown to be the case in Table 3. Although greater than 1, the ratios are less than 3, indicative of pure and high viability cultures.

Molecular basis for limits of detection (LOD) in PCR and immunoassay:

The analysis techniques of PCR and immunoassay are fundamentally different. Polymerase chain reaction (PCR) assay is an amplification technique whereby the target nucleic acid sequence is replicated (copied) until the number of copies reaches a threshold level within the assay reaction mixture. The replication process is exponential, that is, the copy number approximately doubles after each cycle. The LOD of a PCR assay is the minimum number of target sequences that will initiate replication of the target sequence. Immunoassay uses molecular recognition elements (MRE), usually antibodies, to search out and bind to target analyte molecules (often proteins or carbohydrates) in the sample. The assay is designed so that the binding event of MRE to target molecule generates a signal. The greater the number of targets present in the sample, the greater the signal. The limit of detection (LOD) occurs when the cumulative signal of all the MRE-target interactions is greater than the assay background, i.e., blank sample (no target) plus MRE reagents.

In Table 5 the LOD for *Y. pestis* determined by PCR (T-Cor4 instrument) was 13 CFU/mL (0.25 mL sample) or approximately 3–4 CFU per sample. Based on the GE/CFU ratio of 2.38 (see Table 3), 4 CFU is about 10 target sequences in the sample (4 CFU x 2.38

GE/CFU = 9.5 CFU). The LOD for *Y. pestis* determined by immunoassay (Cartridge Reader instrument) was 1×10^5 CFU/mL or 25,000 CFU per 0.25 mL sample. Thus the resolving power of PCR, in terms of CFU, was greater than immunoassay for *Y. pestis*, even though a single *Y. pestis* cell would likely contain 100s or more copies of the target molecule. Table 5 also contains immunoassay data for purified BoNT/A which is a discrete molecule. The LOD for BoNT/A (Cartridge Reader or NIDS instruments) was in the range of 100 ng/mL or 25 ng for a volume of 0.25 mL. Based on a molecular weight of 150 kDa [A.10], the LOD in terms of number of molecules of BoNT/A can be calculated.

$$\begin{aligned} &\text{Number of molecules} \\ &= \{(100 \text{ ng/mL} \times 0.25 \text{ mL}) \div (1.50 \times 10^5 \text{ g/mole})\} \times (6.02 \times 10^{23} \text{ molecules/mole}) \\ &= 10^{11} \text{ molecules.} \end{aligned}$$

Published reports for detection of BoNT employing highly sensitive immunoassay methods indicated LODs in the range of 10^7 to 10^8 molecules per 0.25 mL sample [A.11]. More recent ultrasensitive immunoassays are able to detect BoNT at 10^4 to 10^5 molecules per sample [A.12]. As shown for *Y. pestis*, under ideal or favorable assay conditions (T-COR4, Table 5), the resolving power of PCR is usually greater than immunoassay. In PCR LODs in the order of 10^1–10^2 target sequences (molecules) are readily achievable. For immunoassays the LODs are at best 10^4–10^5 molecules.

Discussion: In the BTB evaluations of bioidentification instruments, reagents from the Critical Reagents Program were used because of their high quality, provenance and availability (to US Army). All of the instruments were tested against the same panel of standard analytes thereby allowing comparisons of the analytical performance of the instruments. If CAF were to undertake or sponsor a test bed evaluation of bioidentification systems, CRP reagents might not be available. Furthermore even if CRP reagents were available, the sample lots, the concentrations and other indicators (shown in Table 3) might not be the same. So the LOD performance of the same instrument with different lots of *B. anthracis* analyte might be different. In other words, using the same standard analytes within a test bed evaluation allows comparison of the LOD performance of the instruments tested with the standard analytes. However it does not allow direct LOD performance comparison of instruments (even the same instrument) to evaluations done with different lots of the same analytes. If the situation arises where different reagent preparations and different samples lots of analyte are used, then having the reagents characterized according to Table 3 plus applying the calculation methods given herein, would allow useful comparisons to be made of one test bed evaluation to another.

In addition to LOD, other analytical performance factors are accuracy and precision. Briefly, accuracy is the conformity of a result to an accepted standard value (reference accuracy) or to a true value. Precision of an assay reflects the experimental (or calculated) result with standard deviation [A.13]. The accuracy and precision are highly dependent upon sample preparation methods, operator skill and the degree of difficulty of the particular instrument. Overall the analytical performance will be affected by how well the operators interface with the hardware system and the sample preparation methods.

For deployed bioidentification of real-world samples, the selectivity of the assays must be considered. Selectivity can be defined as the extent to which an assay method can measure a particular analyte without interference from other components in the sample mixture, such as molecular (or biological) species similar to the analyte (e.g., measuring *B. anthracis* in the

presence of other bacillus species) or molecular species non-related to the analyte (e.g., interferents in the sample matrix). The selectivity of an assay (especially when performed on a commercial instrument) is usually more attributable to the sample preparation, assay protocols and molecular recognition elements used in the assay and less to the instrument hardware. Selectivity in a test bed can be addressed by running the assays in conjunction with a panel standard interferents such as sand, soil, hydrocarbon materials and extraneous biological materials. In situations where the selectivity of an assay is a problem that requires fine-tuning of some aspects of the assay (e.g., modifications in sample preparation or improvements in molecular recognition elements), then the flexibility and openness of the instrument will affect performance and utility to CAF. In field analysis the selectivity problem can be ameliorated through deployment of orthogonal technologies. PCR and immunoassay each approach the problem differently, so that in principle an analysis problem in one technology could be no-problem in the other.

Table A.1: Major molecular components of bacterial cells and viruses.

<table>
<tr><th rowspan="2">Component</th><th colspan="2">Bacteria</th><th>Virus</th></tr>
<tr><th>% total weight wet [A.14]</th><th>% total weight dry[A.15]</th><th>% total weight dry [A.15], [A.16]</th></tr>
<tr><td>Water</td><td>70</td><td>–</td><td>–</td></tr>
<tr><td>Proteins</td><td>15</td><td>55</td><td>60–80</td></tr>
<tr><td>DNA</td><td>1</td><td>3</td><td rowspan="2">5–40
(DNA or RNA)</td></tr>
<tr><td>RNA</td><td>6</td><td>21</td></tr>
<tr><td>Carbohydrates</td><td>3</td><td>5</td><td>0–6</td></tr>
<tr><td>Lipids</td><td>2</td><td>12</td><td>0–30</td></tr>
<tr><td>Metabolites</td><td>2</td><td rowspan="2">4 (small molecules + ions)</td><td>–</td></tr>
<tr><td>Inorganic ions</td><td>1</td><td>–</td></tr>
</table>

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Appendix 1 Scoring bars and vendor contact information

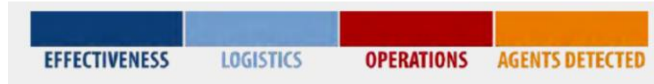

Figure AP.1-1a: Description of Scoring Bar for CBR Technological Survey. The four colours represent the top-level criteria shown in Figure 1 of this report (from Reference 1 p. 5).

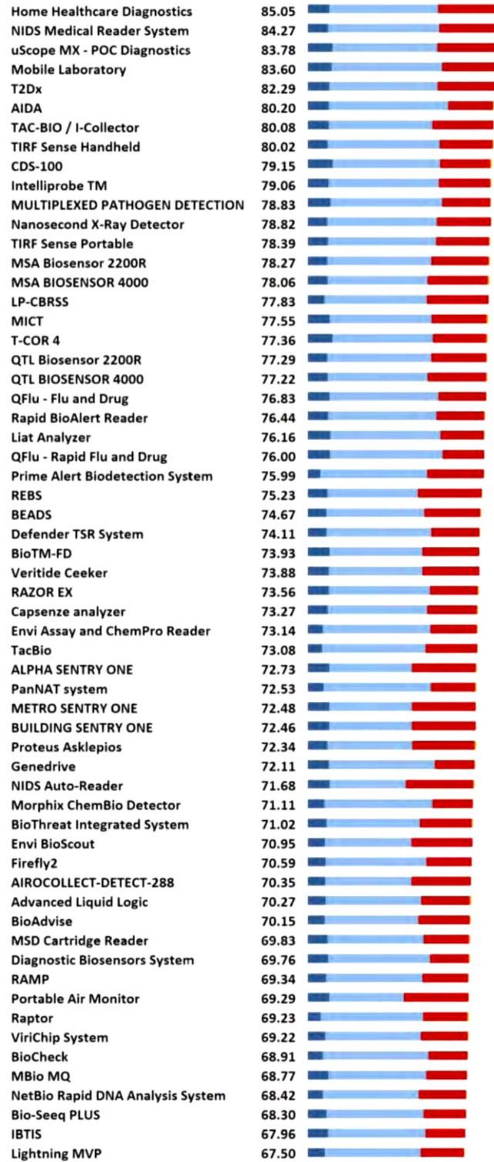

Figure AP.1-1b: Field-use rankings of biological specific systems for CBR Technological Survey (from Reference 1 p. 8). See Reference 1 for system details.

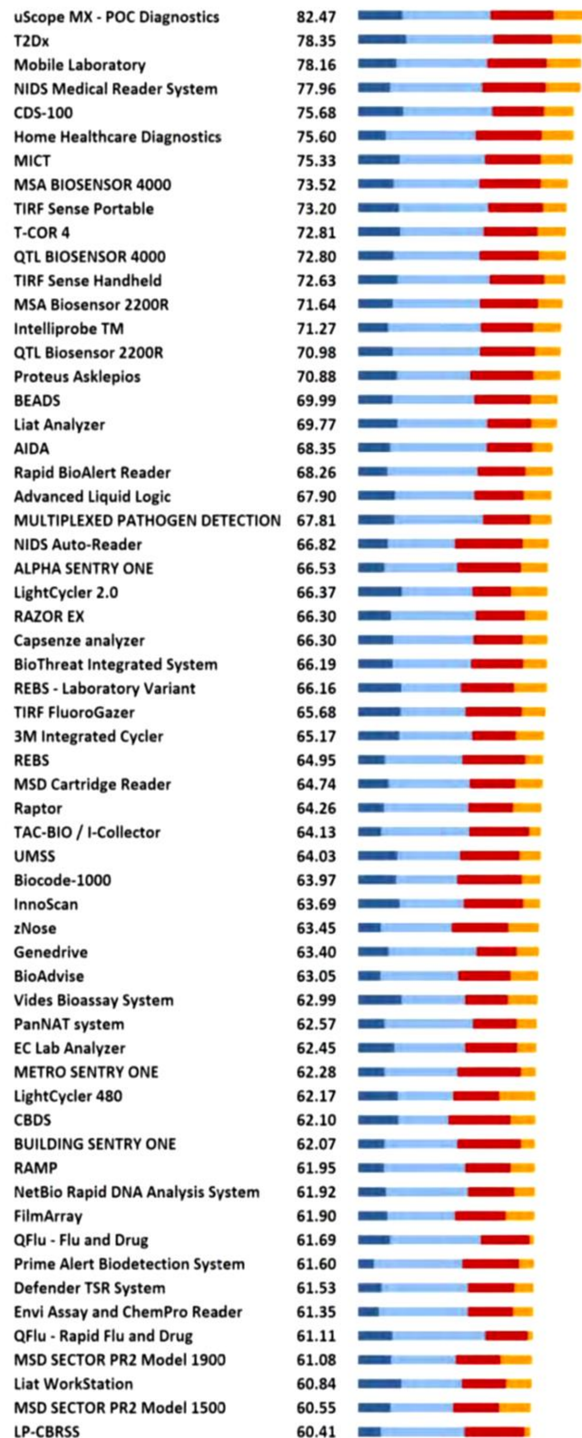

Figure AP.1-1c: Mobile Laboratory Rankings of biological specific systems for CBR Technological Survey (from Reference1 p. 12). See Reference 1 for system/instrument details.

Table AP.1-1: List of instruments systems and vendor contacts for the Biosensors Test Bed.

System / instrument	Vendor	Contacts	Notes
FilmArray	BioFire Diagnostics Inc.	390 Wakara Way Salt Lake City UT 84108 USA	Email: support@biofiredx.com Tel. 801-736-6354
Liat (now available as cobas®Liat)	IQuum (now Roche Diagnostics)	Roche Diagnostics Canada 201 Armand-Frappier Blvd, Laval QC Canada H7V 4A2	www.rochecanada.com Email: canada.webmaster@roche.com Tel: 800 361 2070
RAZOR EX	BioFire Defense	79 W 4500 S, Suite 14 Salt Lake City, UT 84107 USA Tel: 801-262-3592	RAZOR EX BioDetection System info@biofiredefense.com Email: support@biofiredefense.com
T-COR 8™	Tetracore Inc.	9901 Belward Campus Drive Suite 300, Rockville, MD 20850 USA Tel: 240-268-5400	T-COR 8™ Real-time PCR Thermocycler www.tetracore.com/index.html
Genedrive by Epistem	Epistem Ltd	UK: 48 Grafton Street, Manchester M13 9XX United Kingdom Tel: +44 (0) 161 606 7258 www.epistem.co.uk	USA: Epistem Inc., One Broadway Cambridge, MA 02142 USA www.genedrive.com Email: info@epistem.co.uk
Cartridge Reader	Meso Scale Devices	1601 Research Blvd. Rockville, MD 20850-3173 USA Tel: 240-314-2600	Email: customerservice@mesoscale.com Scientific/Technical Support: Tel: 240-314-2798
ANP NIDS®	ANP Technologies Inc.	824 Interchange Blvd Newark, DE 19711 USA Tel: 302-283-1730	ANP NIDS® High Throughput Screening E-mail: info@anptinc.com
SPIRIT Portable SPR	Seattle Sensor Systems	1311 Republican St Seattle, WA 98109 USA Tel. 206-588-1927	SPIRIT Portable SPR Instrument Information requests: sssinfo@seattlesensors.com
SpinDx™	Sandia National Laboratory	Livermore, CA USA www.sandia.gov/research/research_foundations/bioscience	SpinDx™ Point-of-Care Diagnostics
MAGPIX®	Luminex Corp	12212 Technology Blvd, Suite 130 Austin, TX 78727 USA Tel: 512-219-8020	Technical support Tel: 512-381-4397
RAPTOR	Research International Inc.	17161 Beaton Road SE Monroe, WA 98272-1034 USA Tel: 360-805-4930	www.resrchintl.com/RAPTOR_Bioassay_System.html

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Appendix 2 Description of assays

Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a molecular biology technique that is widely employed to amplify specific sequences of DNA over many orders of magnitude.

The PCR process requires:

- double stranded DNA template;
- 2 DNA primers, one that is complementary to the 5'-end of the "sense" strand and another complementary to the 3'-end of the "antisense" strand;
- the enzyme, DNA polymerase; and
- deoxynucleoside triphosphates, dA, dT, dG, dC, which are incorporated into the amplified DNA product.

The PCR reaction is usually carried out in reaction tubes at a volume of 10–100 µL in a thermal cycling instrument. The thermal cycling process heats the reaction mixture to denature the DNA. On cooling the primer binds to the target sequence (also referred to as the template). The DNA polymerase enzyme catalyses the extension (i.e., elongation) of the bound primer molecule with deoxynucleosides complementary to the template, effectively copying the template in antisense. After elongation, the cycle is repeated (heal, cool, elongation) usually about 10–30 times. The amount of copied template increases exponentially during the thermal cycling phase of the assay.

Further information can be found at [Ap.2-1].

Immunoassay

Immunoassay is a biochemical technique that uses antibody-mediated interactions to detect and measure biological molecules. During the past 40 years many immunoassay formats have been developed for thousands of analytes. However essentially all immunoassays are based on two phenomena, 1) the high variety and high affinity of antibodies in the molecular recognition process and 2) the ability of chemical and biochemical labels to generate a signal in response to molecular recognition. In most cases an antibody is employed to capture or pull-down the target analyte molecule. A separation step is performed wherein the captured analyte molecule is retained and the non-analyte material of the sample is washed away. After separation a second antibody, possessing a signal-generating label, is added to the assay and subsequently binds to the captured analyte. Excess second antibody is washed away. The resulting signal emanating from the antibody-analyte complex is indicative of the analyte in the sample.

Further information can be found at [Ap.2-2].

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Appendix 3 Critical Reagents Program: agents and reagents

Target panel

The specific agents acquired from the JPEO Critical Reagents Program were:

- *Bacillus anthracis* Ames (Gram-positive spore-forming bacilli) [Ap.3-1]–[Ap.3-3];
- *Yersinia pestis strain* CO92 (Gram-negative rod-shaped bacterium) [Ap.3-4], [Ap.3-5];
- Vaccinia (double-stranded deoxyribonucleic acid (dsDNA) Orthopox virus, Smallpox [*Variola*] stimulant) VAC strain Elstree (Lister) [Ap.3-6]–[Ap.3-8];
- Venezuelan equine encephalitis virus (+sense single-stranded RNA virus, an Alphavirus) VEE virus vaccine strain TC-83 [Ap.3-9], [Ap.3-10];
- *Clostridium botulinum* Type A neurotoxin (BoNT A; protein toxin);

BoNT A was supplied by Metabiologics, Inc. (Madison, WI) as the active holotoxin complex. The concentration of the toxin was 1 mg/mL with a specific toxicity of $3.5x10^7$ (MLD_{50} /mg). The A260/278 ratio of the toxin product was determined by the producer to be less than 0.55, indicative of a preparation that has low DNA contamination. [Ap.3-11], [Ap.3-12].

Antibodies used in Biosensors Test Bed evaluations

The antibodies were acquired from JPEO Critical Reagents Program:

- anti-B. anthracis monoclonal antibody (Cat Num: AB-BA-MAB4, Lot Num: R0178);
- Goat anti-B. anthracis antibody (Cat Num: AB-G-BA, Lot Num: PGGG016);
- anti-*Y. pestis* monoclonal antibody (Cat Num: AB-YERS-MAB1, Lot Num: R0183);
- Rabbit anti-*Y. pestis* antibody (Cat Num: AB-R-YERS, Lot Num: J040400-01);
- anti-VAC monoclonal antibody (Cat Num: AB-VACC-MAB2, Lot Num: J-191101-01);
- Rabbit anti-VAC antibody (Cat Num: AB-R-VACC, Lot Num: 080205-01);
- anti-VEE monoclonal antibody (Cat Num: AB-VEE-MAB2, Lot Num: 220711-01);
- anti-VEE monoclonal antibody (Cat Num: AB-VEE-MAB3, Lot Num: J-291002-01);
- anti-BoNT A monoclonal antibody (Cat Num: AB-BOT-A-MAB1, Lot Num: 030707-01); and
- anti-BoNT A monoclonal antibody (Cat Num: AB-BOT-A-MAB2 Lot Num: 260607-01).

Commercial products: QIAGEN DNeasy kit - DNA Extraction
QIAGEN Inc. – Canada 181 Bay Street, Suite 4400, Toronto, Ontario M5J 2T3.
Technical support: Phone Number: 800-362-7737, www.qiagen.com.

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Appendix 4 Technology readiness

*Table AP.4-1: Technology readiness levels.**

TRL	Definition	Description	Supporting Information
1	Basic principles observed and reported.	Lowest level of technology readiness. Scientific research begins to be translated into applied research and development (R&D). Examples might include paper studies of a technology's basic properties.	Published research that identifies the principles that underlie this technology. References to who, where, when.
2	Technology concept and/or application formulated.	Invention begins. Once basic principles are observed, practical applications can be invented. Applications are speculative, and there may be no proof or detailed analysis to support the assumptions. Examples are limited to analytic studies.	Publications or other references that outline the application being considered and that provide analysis to support the concept.
3	Analytical and experimental critical function and/or characteristic proof of concept.	Active R&D is initiated. This includes analytical studies and laboratory studies to physically validate the analytical predictions of separate elements of the technology. Examples include components that are not yet integrated or representative.	Results of laboratory tests performed to measure parameters of interest and comparison to analytical predictions for critical subsystems. References to who, where, and when these tests and comparisons were performed.
4	Component and/or breadboard validation in a laboratory environment.	Basic technological components are integrated to establish that they will work together. This is relatively "low fidelity" compared with the eventual system. Examples include integration of "ad hoc" hardware in the laboratory.	System concepts that have been considered and results from testing laboratory-scale breadboard(s). References to who did this work and when. Provide an estimate of how breadboard hardware and test results differ from the expected system goals.
5	Component and/or breadboard validation in a relevant environment.	Fidelity of breadboard technology increases significantly. The basic technological components are integrated with reasonably realistic supporting elements so they can be tested in a simulated environment. Examples include "high-fidelity" laboratory integration of components.	Results from testing laboratory breadboard system are integrated with other supporting elements in a simulated operational environment. How does the "relevant environment" differ from the expected operational environment? How do the test results compare with expectations? What problems, if any, were encountered? Was the breadboard system refined to more nearly match the expected system goals?

TRL	Definition	Description	Supporting Information
6	System/subsystem model or prototype demonstration in a relevant environment.	Representative model or prototype system, which is well beyond that of TRL 5, is tested in a relevant environment. Represents a major step up in a technology's demonstrated readiness. Examples include testing a prototype in a high-fidelity laboratory environment or in a simulated operational environment.	Results from laboratory testing of a prototype system that is near the desired configuration in terms of performance, weight, and volume. How did the test environment differ from the operational environment? Who performed the tests? How did the test compare with expectations? What problems, if any, were encountered? What are/were the plans, options, or actions to resolve problems before moving to the next level?
7	System prototype demonstration in an operational environment.	Prototype near or at planned operational system. Represents a major step up from TRL 6 by requiring demonstration of an actual system prototype in an operational environment (e.g., in an aircraft, in a vehicle, or in space).	Results from testing a prototype system in an operational environment. Who performed the tests? How did the test compare with expectations? What problems, if any, were encountered? What are/were the plans, options, or actions to resolve problems before moving to the next level?
8	Actual system completed and qualified through test and demonstration.	Technology has been proven to work in its final form and under expected conditions. In almost all cases, this TRL represents the end of true system development. Examples include developmental test and evaluation (DT&E) of the system in its intended weapon system to determine if it meets design specifications.	Results of testing the system in its final configuration under the expected range of environmental conditions in which it will be expected to operate. Assessment of whether it will meet its operational requirements. What problems, if any, were encountered? What are/were the plans, options, or actions to resolve problems before finalizing the design?
9	Actual system proven through successful mission operations.	Actual application of the technology in its final form and under mission conditions, such as those encountered in operational test and evaluation (OT&E). Examples include using the system under operational mission conditions.	Operational Test and Evaluation reports.

* Technology Readiness Assessment (TRA) Guidance. Prepared by the Assistant Secretary of Defense for Research and Engineering (ASD(R&E)) United States Department of Defense. April 2011 (PDF). http://www.acq.osd.mil/chieftechnologist/publications/docs/TRA2011.pdf.

List of symbols/abbreviations/acronyms/initialisms

BA	*Bacillus anthracis* (Gram-positive spore-forming bacillus)
BioDIM	biodetection/identification/monitoring
BoNT/A	*Clostridium botulinum* neurotoxin serotype A
bp	base pair
BTB	Edgewood Biosensor Test Bed Hand-held and Man-portable Edition (2013)
CAF	Canadian Armed Forces
CBRTS	Chemical Biological Radiological Technology Survey (2011)
CFU	Colony forming unit
COTS	commercial-off-the-shelf
CRP	Critical Reagent Program
Da	dalton (a unit of atom mass)
DNA	deoxyribose nucleic acid
DND	Department of National Defence
DRDC	Defence Research and Development Canada
ECBC	US Army Edgewood Chemical Biological Center
GE	genome equivalents
GDMS	Global CBRN Detector Market Survey (2014)
k	kilo (prefix for 10^3)
LOD	limit of detection
M	mega (prefix for 10^6)
MSD	Meso Scale Discovery
MOTS	military-off-the-shelf
PCR	polymerase chain reaction

PFU	plaque forming unit
SI	International Systems of Units
SME	subject matter expert
TRL	technology readiness level
TRA	technology readiness assessment
VAC	Vaccinia *variola* Elstree (Lister) strain
VEE	Venezuelan equine encephalitis virus
YP	*Yersinia pestis* (Gram-negative bacterium)

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The present report is a review and analysis of the operational performance of mobile laboratory and field-use identification systems for biological threat agents. The main source for the review and analysis was the *Edgewood Biosensors Test Bed Hand-held and Man-portable Edition* (2013) plus market surveys of commercial systems published by US Department of Defense (2011 and 2014). The instruments or systems evaluated in the *Edgewood Biosensors Test Bed* and the surveys were commercial off-the-shelf instruments or prototypes at an advanced stage of development. The focus of the *Edgewood Biosensors Test Bed* was the performance of the instruments in the hands of laboratory scientists and trained military field operators. For the hands-on evaluation 11 systems based on nucleic acid (polymerase chain reaction) or antibody-based (immunoassay) technology were downselected. The *Edgewood Biosensors Test Bed* showed the importance of conducting the evaluations in a real-world setting, i.e., actually taking field-use instruments to the field with military operators. The purpose of the present report is to provide project managers in the Canadian Armed Forces (CAF) with an outline for making decisions on acquisition of bioidentification instruments and systems. The report also provides guidance for project managers on how to conduct evaluations of candidate instruments. Because of the varied operations of CAF personnel, no single instrument will likely meet the requirements of all deployments. Several of the highly rated instruments in the *Edgewood Biosensors Test Bed* are potential candidates for use by the CAF. The main conclusion of the report is that assessment of bioidentification instruments for CAF operations is a difficult but tractable problem. The report contains a strategy to dissect the problem and achieve meaningful knowledge that can be applied to a procurement process.

Le présent rapport porte sur l'examen et l'analyse de la performance opérationnelle des laboratoires mobiles et des systèmes d'identification sur le terrain des agents de menace biologique. Pour les besoins de l'examen et de l'analyse, nous avons utilisé principalement le modèle portatif du banc d'essai de biocapteurs Edgewood (2013), ainsi que des études de marché sur des systèmes commerciaux réalisées par le département de la Défense des États-Unis (2011 et 2014). Les systèmes évalués avec le banc d'essai de biocapteurs Edgewood et les études de marché étaient des instruments ou des prototypes commerciaux à un stade avancé de développement. Nous avons utilisé le banc d'essai dans le but d'évaluer la performance des instruments entre les mains de chercheurs de laboratoire et d'utilisateurs militaires exercés sur le terrain. Pour l'évaluation pratique, nous avons sélectionné 11 systèmes fondés sur une technologie à base d'acide nucléique (réaction en chaîne de la polymérase) ou d'anticorps (immuno-essai). L'utilisation du banc d'essai de biocapteurs Edgewood a révélé l'importance de mener les essais en situation réelle, c'est-à-dire en demandant à des utilisateurs militaires d'utiliser les instruments sur le terrain. Le présent rapport a pour but de fournir aux gestionnaires de projets des Forces armées canadiennes (FAC) un cadre de prise de décision concernant l'acquisition d'instruments et de systèmes de bioidentification. Il fournit également aux gestionnaires de projets des lignes directrices pour l'évaluation des instruments d'intérêt potentiel. En raison de la nature variée des opérations des FAC, il est probable qu'aucun instrument ne réponde aux besoins de tous les

déploiements. Plusieurs des instruments les mieux cotés par le banc d'essai des biocapteurs Edgewood sont susceptibles d'être utilisés par les FAC. La conclusion principale du rapport est que l'évaluation d'instruments de bioidentification destinés aux opérations des FAC est un problème complexe mais soluble. Le rapport propose une stratégie pour disséquer ce problème et ainsi acquérir un savoir considérable et applicable aux processus d'approvisionnement.

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Bioidentification; biosenors; immunoassay; pcr.