EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1201

EDGEWOOD BIOSENSORS TEST BED HAND-HELD AND MAN-PORTABLE EDITION

Janet Betters
Robert Dorsey
Peter Emanuel

RESEARCH AND TECHNOLOGY DIRECTORATE

Bryan Rivers
Eric Schaffer

SCIENCE APPLICATIONS INTERNATIONAL CORPORATION, INC.
Gunpowder, MD 21010-0068

Evan Skowronski

TMG BIOSCIENCES
Incline Village, NV 89451

September 2013

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
XX-09-2013	Final	Mar 2012 – Sep 2013

4. TITLE AND SUBTITLE
Edgewood Biosensors Test Bed
Hand-held and Man-Portable Edition

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Betters, Janet; Dorsey, Robert; Emanuel, Peter (ECBC); Rivers, Bryan; Schaffer, Eric (SAIC); and Skowronski, Evan (TMG Biosciences)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRB-S, APG, MD 21010-5424
SAIC, P.O. Box 68, Gunpowder, MD 21010-0068
TMG Biosciences, 774 Mays Blvd. #10-455, Incline Village, NV 89451

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1201

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Joint Program Manager-Medical Countermeasures Systems
1564 Freedman Drive
Fort Detrick, MD 21702-9226

10. SPONSOR/MONITOR'S ACRONYM(S)
JPM-MCS

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

This Biosensors Test Bed Report was funded by the Department of Defense Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD) Joint Program Manager (JPM) - Transformational Medical Technologies (now JPM– Medical Countermeasure Systems) to provide program managers and other acquisition decision makers with a comprehensive snapshot of commercially available biosensors that may be applied to biological agent detection and identification. Importantly, the actual performance of the systems in the hands of both dedicated laboratory personnel and field end-users was assessed. Commercial off-the-shelf and advanced concept biological agent identifiers were directly compared against a broad set of conditions and criteria in order to benchmark the current state of biodetection hardware. In addition, we hope this study provides data for existing and future DoD acquisitions and is applicable in biodefense programs throughout the United States Government. The information in this report was gathered using online surveys completed by the vendors, laboratory testing, and end-user assessments in relevant field environments. The data was analyzed using an adjustable scoring matrix specific to the needs of JPEO-CBD.

15. SUBJECT TERMS

Biosensors	Identifier	Antibody	Technical readiness level
Nucleic acid	Surveillance	Handheld	Mobile laboratory
Target	Bacteria	Toxin	Man portable
Virus	Assay		

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Renu B. Rastogi
U	U	U	UU	210	**19b. TELEPHONE NUMBER** *(include area code)* (410) 436-7545

Blank

PREFACE

The work described in this report was started in March 2012 and completed in September 2013.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for the purposes of advertisement.

The text of this report is published as received and was not edited by the Technical Releases Office, U.S. Army Edgewood Chemical Biological Center.

This report has been approved for public release.

Blank

Edgewood
Biosensors Test Bed
Hand-held and Man-portable Edition

September 2013

Public Release - Unclassified

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

Dear Reader:

This *Biosensors Test Bed Report* was funded by the Department of Defense Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD) Joint Program Manager (JPM) - Transformational Medical Technologies (now JPM – Medical Countermeasure Systems) to provide program managers and other acquisition decision makers with a comprehensive snapshot of commercially available biosensors that may be applied to biological agent detection and identification. Importantly, the actual performance of the systems in the hands of both dedicated laboratory personnel and field end-users was assessed. Commercial off-the-shelf and advanced concept biological agent identifiers were directly compared against a broad set of conditions and criteria in order to benchmark the current state of biodetection hardware. In addition, we hope this study provides data for existing and future DoD acquisitions and is applicable in biodefense programs throughout the United States Government.

The information in this report was gathered using online surveys completed by the vendors, laboratory testing, and end-user assessments in relevant field environments. The data was analyzed using an adjustable scoring matrix specific to the needs of JPEO-CBD. This scoring matrix was designed to assess Sensitivity, Multiplex Capability, Physical Attributes, Cost, Power Requirements, and Technical Readiness. Each attribute was weighted using an adjustable scale which allows decision makers to adjust scores based on the needs of their programs.

The report is organized into sections that can be easily understood and stand alone. The "Test Bed Summaries" section is a quick reference guide made up of two page synopses for each of the 11 systems tested. The majority of the report is split into two section categories: nucleic-acid (blue) or an antibody-based (red) technology. Within each of these sections, the systems are listed alphabetically by company name. Data from laboratory testing is included followed by a summary that discusses the technology and assay design and any insight into the performance of the system from the perspective of Subject Matter Experts in the field. The Mobile Laboratory and Field Test Assessments are each discussed in their own sections with user feedback consolidated in easy to read tables for each of the systems. Our final section is a review of the overall scores for each attribute along with a Discussion Section that summarizes our efforts and limitations of the study. We hope that you find this report both helpful and interesting.

Sincerely,

Scott ReMine
Program Manager
JPM-Medical Countermeasure Systems
JPEO-Chemical Biological Defense
scott.w.remine.civ@mail.mil

Janet Betters
Project Manager
BioSensors Branch
Edgewood Chemical Biological Center
janet.l.betters.civ@mail.mil

CONTRIBUTORS

ECBC BioSciences Division
Janet Betters
Carollynn Goldburn
Catherine Sabol
Robert Dorsey, BioSensors Branch Chief
Peter Emanuel, PhD, BioSciences Division Chief

SAIC
Amy DeKett
Joseph Insalaco
Bryan Rivers
Eric Schaffer, PhD

STC
Erin Norton

EXCET
Kristy Williams

ECBC Advanced CBRNE Training Team
Carrie Poore, PhD
Marcus Thermos

US Army Joint Program Executive Office
David Klaasse
Brady Redmond, PhD
Scott Remine
Martina Siwek, PhD
Michael Smith, MPhil, PhD, PMP

Booz Allen Hamilton, Belcamp, MD Office

Tauri Group
Kenneth Tucker
Randy Vines

JRAD
Jeff Morgan

TMG, Incorporated
Evan Skowronski, PhD

US Army 20th Support Command

US Army 22nd Chemical Battalion

US Army 56th Chemical Reconnaissance Detachment, 5th Special Forces Group (Airborne)

EXECUTIVE SUMMARY

Over the last decade, the Edgewood Chemical Biological Center (ECBC) conducted numerous surveys of technologies. Until now, these surveys relied on manufacturer supplied information without a laboratory or field assessment to corroborate the system performance. The Biosensors Test Bed offers a unique opportunity to assess system performance in an International Organization for Standardization (ISO) 17025-compliant laboratory and in operationally relevant real-world settings.

Over an 18 month period, ECBC scientists partnered with soldiers to systematically down-select systems identified through market surveys and open data calls. Beginning with 40 biological agent identifiers, the Test Bed staff chose the top 16 candidate systems for secondary assessment. The goal during this down-selection was to identify systems that would represent a diverse collection of detection methodologies, with consideration of the stage of development, assay availability, and logistics. Inability to acquire a working system or commercial unwillingness to participate reduced the number of candidate systems to 12 as the Test Bed moved into the laboratory assessment. Overall, the laboratory scientists and field operators agreed that the standout devices were the antibody-based MesoScale Diagnostics Cartridge Reader, the IQuum Lab in a Tube (Liat™) polymerase chain reaction (PCR) device, and the BioFire™ Diagnostics FilmArray® PCR device. While each of these devices would benefit from more portability and minor re-engineering, user ratings indicated these devices were user-friendly and reliable. On the other end of the spectrum, the Sandia National Laboratories' SpinDx™, Seattle Sensors Systems' SPIRIT™ and the Research International RAPTOR scored poorly and assessed to be unreliable and difficult to use.

The value of laboratory testing complemented by field utility assessments was evident with these lower-scoring systems. Though sensitivity and reliability issues were encountered in the laboratory, operators were sometimes able to overcome these issues by working with the company, rebooting, or flushing the devices. However, in the field when the operators encountered system failures, fluidic clogs, or software errors they were unable to rectify these issues and the exercise failed.

Several devices scored well because of their small size and simplicity, but will require re-engineering to increase applicability to military environments. About the size of a paperback book and operated with a single button, the Epistem Genedrive™ PCR device is an example of this group. The device was created for a clinical diagnostic market and showed great potential as a low-cost small-footprint highly sensitive biological identifier in the laboratory; however, significant re-engineering will be required to make this device conducive to field use. Because small size is a top priority for many military users, the ANP NanoIntelligent Detection System (NIDS®) with Stand Alone Reader is a device currently being used by some in the field. However, in this assessment, although the NIDS was easy to use, it was plagued with sensitivity and specificity issues typical of other hand-held antibody-based assay systems. On the other end of the size spectrum is the Luminex MAGPIX®, which is slightly larger than a computer tower with an accompanying laptop. The MAGPIX is an antibody-based system that has high assay flexibility with the potential for highly multiplexed and high throughput analysis. In order for the system to be of military utility in the field, it will require significant re-engineering. As it stands right now, the MAGPIX is a reliable laboratory based instrument.

The Biosensors Test Bed clearly demonstrated the value of conducting both laboratory analyses and a field based assessments. Table top assessments based on manufacturer supplied data tell only half the story and many devices that appeared favorable on paper were revealed to have flaws and areas requiring restructuring when taken to the field. In closing, it is important to note that the diverse needs of the warfighter mean that no single device will fulfill all missions and programmatic requirements.

INTRODUCTION

Biological detection systems have benefited from the progress made in a variety of scientific and engineering disciplines. The development and use of sophisticated, often multiplexed, molecular biology assays along with engineering developments such as inexpensive light-emitting diode (LED) illumination, charge-coupled device (CCD) imaging, high–speed computer processing, and lightweight batteries illustrate this technical convergence. With the application of these advances, the current generations of detection systems are faster, have a greater sample throughput, and produce far more data than their predecessors. In addition to performance improvements, there is now a greater diversity of detection systems than ever before. Commercially available platforms vary in size, form, cost, and ease-of-use.

Despite this variety of technologies, there is no ideal system that can meet the wide range of mission requirements and programmatic objectives within the federal government. It is essential for program managers and other decision makers to explore the available options prior to making development and acquisition investment decisions. For biological detection systems investments, an analysis of alternatives should include not only a comprehensive characterization of the available platforms, but also 3rd party validation of manufacture claims. This type of thorough evaluation not only serves to match the appropriate system to a program or operational scenario, but it can identify platforms to provide common solutions for similar federal interagency initiatives.

The purpose of this study was to provide an unbiased technical evaluation of candidate biological detection systems in support of future Department of Defense (DoD) and interagency acquisition decisions. Because the effort wasn't tied to an acquisition program, this study had the flexibility to examine a wide variety of performance criteria to benefit diverse federal initiatives. As a result, this study is unique for several key reasons. The first distinguishing feature is that it evaluated systems in both an ISO 17025 accredited laboratory setting with highly skilled scientists as well as in a field environment with end-users. System performance can dramatically change under different operating environments, while the routines and habits of trained biologists in a laboratory setting compared to a responder or other end user in a field setting may also impact test results. Furthermore, safety equipment, lack of laboratory infrastructure and field operating procedures can affect or even prevent a system from being used as the manufacture intended. A second unique aspect of this study is that it not only systematically categorizes and ranks the attributes of biological detection systems, but it also scores them using an adjustable performance scoring matrix. Thus, the weights of the scoring can be adapted to fit different programmatic requirements. The expectation is that these amendable results will reveal instances where the same device may be used for different programs with overlapping goals and requirements. This may lead not only to leveraged funding, but to a more cohesive strategy for biothreat detection.

Importantly, the results of this study favored smaller devices that are suited to, or have the capability to be developed into a device that may be used in a field environment. It is also crucial to note that some of the tested systems are still being developed and that this study represents just a snapshot of their performance. This study could serve as the basis for follow-on evaluations as these devices evolve and mature.

BACKGROUND

The DoD JPEO-CBD's JPM-TMT (now part of JPM-MCS) conducted a technical information survey in early 2012 and subsequent hands-on evaluations of existing biological agent identifiers. An open Request for Information (RFI) was issued with the intent of soliciting input from industry and federal laboratories to identify existing equipment capable of field- and laboratory-based pathogen identification. The objective of this report is to provide a snapshot of commercially available biosensor systems that may be applicable for biological agent detection and identification. This report not only provides a summary of the physical characteristics of these systems, but it details the performance of these systems in both laboratory and field settings.

In contrast to the plethora of biological identifier instruments available to dedicated stationary diagnostic or research laboratories, the number of such systems dedicated, or even applicable, to man-portable field environments or mobile laboratories is somewhat limited. The 40 responses to the RFI, which included biological agent identifiers in stages of development from concept to commercial off-the-shelf (COTS) instruments, were reviewed and initially sub-divided into the following categories based on size and detection technology:

- Handheld/Man-portable – Antibody-Based
- Handheld/Man-portable – Nucleic Acid-based
- Mobile Lab – Antibody-based
- Mobile Lab – Nucleic Acid-based

Information submitted in response to the RFI was the foundation for this subdivision and subsequent characterization; however, other relevant sources of information were also utilized (e.g., Emanuel, P. & Caples, M. (2011) The Chemical, Biological, Radiological Technology Survey). Information gathered by other ongoing United States Government (USG) technology systems evaluations, including the Defense Threat Reduction Agency's "24 Month Challenge" and the JPEO-CBD's Common Analytical Laboratory System, were also utilized for the initial assessment of biological identifiers.

A down-selection of the instruments by ECBC and JPM-TMT staff generated a list of 16 candidate systems for a secondary assessment. The goal during this down-selection was to identify systems that would represent a diverse collection of detection methodologies, with consideration given to stage of development, assay availability, and logistics. Selection of nearly duplicate systems was avoided so that the assessments could focus on distinct technologies. After further assessment and characterization, twelve candidate systems were chosen for inclusion in the formal test bed assessment. Candidate technologies were evaluated for their ability to detect known samples in an ISO 17025 compliant laboratory at ECBC in order to document performance and verify vendor claims. The instruments were directly compared by challenging them with identical preparations of inactivated biological threat agents.

In addition to the comprehensive laboratory assessment performed by skilled personnel, the systems were evaluated by end-users in several operationally relevant environments. Specifically, the field assessments were conducted by operators from the Army's 5th Special Forces Group (Airborne), the Army's 22nd Chemical Battalion and the Army's 20th Support Command. The field assessments were conducted at ECBC's Skippers Point, a non-laboratory site of former military housing at Aberdeen Proving Ground that is now used to simulate operational situations, and within the 20th Support Command's Heavy Mobile Expeditionary Laboratory (HMEL).

Figure 1. Members of the Army 5th Special Forces Group (Airborne) perform sample analysis using the BioFire RAZOR EX at ECBC's Skippers Point operation simulation site.

Figure 2. A member of the Army 22nd Chemical Battalion (right) analyzes a sample using the Research Internal RAPTOR under the watchful eye of an ECBC scientist at Skippers Point.

Figure 3. The Army 20th Support Command's HMEL at Aberdeen Proving Ground served as the site for the biological agent identifiers' mobile laboratory assessments.

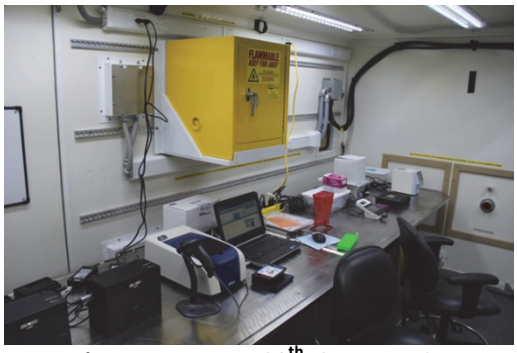


Figure 4. Army 20th Support Command HMEL interior.

Performance data and user input were captured in data sheets and questionnaires and incorporated into multiple scoring matrices, which allowed for the comparison of identification systems. For rapid and easy reader assessment, this report presents a two-page summary of the evaluation of each biological agent identifier system at the beginning of the laboratory evaluations. The reviewers also assigned Technology Readiness Levels (TRL) to each system, based on DoD guidance (Appendix A). Acquisition managers will be able to triage individual and classes of detection systems with high-confidence performance data, weighting the data as appropriate to satisfy specific program requirements. This report is expected to support existing and future JPEO-CBD, DoD, and other USG acquisition efforts, and could be expanded to include other surveillance technologies as appropriate. This report complements other reviews of contemporary technologies to counter threats from weapons of mass destruction, including The Chemical, Biological, Radiological Technology Survey (2011; Emanuel, P. & Caples, M.); The 2013 Nucleic Acid Purification Market Survey and CBRN Sample Preparation Horizon Scan (Betters, J., Emanuel, P. & Caples, M.); and The 2013 Global CBRN Detector Market Survey and Horizon Scan (Emanuel, P. & Caples, M.), the covers of which are shown in Figure 5.

Figure 5. Global Horizon Scan Surveys

IDENTIFICATION AND DOWN-SELECTION OF SYSTEMS

JPM-TMT RFI

The JPEO-CBD JPM-TMT (now part of JPM-MCS) office issued an RFI, "Development of a Handheld Pathogen Identification and Characterization System," on FedBizOpps.gov that sought information regarding existing biological agent identifier technologies. The solicitation requested information regarding any "lightweight, easy to use, handheld bio-identification system to provide the frontline warfighters with the ability to identify and characterize pathogens." The RFI solicited specific information for systems that perform:

1. Analysis to include pathogen characterization and identification, and the potential to identify chemical agents
2. Sample preparation to be used prior to a sample analysis on a handheld device

These two capabilities would ideally function as an integrated, end-to-end system or as separate technologies with compatible characteristics. The companies were asked to provide details on their technologies, in the form of these inquiries:

1. Describe the technology platform
2. What is the multiplex capability of the technology?
3. What assays have been developed for the platform (e.g., infectious disease agents, biowarfare agents)?
4. How have the assays been tested and validated?
5. What is the assay run time?
6. Does the device have the capability to discriminate between biological variants?
7. Does the device identify or distinguish threat characteristics (e.g., antibiotic resistance, increased virulence)?
8. What is the sensitivity and specificity of the assays?
9. How much sample is required for testing?
10. Is sample preparation an integrated part of the analysis? What are the requirements for the prepared sample? Is the raw or prepared sample preserved?

11. What samples / biologics can be assayed (e.g., spores/cysts, Gram-positive and Gram-negative bacteria, viruses)?
12. What types of matrices can be analyzed (e.g., blood, sputum, water, surface samples, and aerosol samples, environmental)?
13. Is the technology capable of identifying chemical warfare agents, toxic industrial chemicals, or toxic industrial materials?
14. What are the power requirements of the technology? Is it a mobile device? If battery powered, how many runs per single battery charge?
15. What support equipment is required? Consumables? Ancillary sample preparation equipment?
16. Under what environmental conditions has the technology been tested?
17. If greater than 3lbs, how much development time would be needed to reduce the bio-identifier to less than three lbs. including power source?
18. How is the analysis data presented to the user? Does the technology have data transmission capability (e.g. internet, satellite, mobile networks, direct interface with a communications device)?
19. Does the technology have geolocation capability?
20. What is the current unit cost? Sample analysis cost?
21. Does the device require calibration? What are system maintenance requirements?

Besides the companies that responded to the RFI, companies with potentially applicable technologies were notified of this opportunity and encouraged to submit. Ultimately, 40 submissions were received, of which 30 submissions described a biological agent identifier technology developed to at least a "working prototype" level of maturity. Eight of the submissions were proposed or conceptual designs and two were exclusively sample preparation/collection devices.

Down Selection of Candidate Systems

A selection panel consisting of staff from ECBC and JPEO-CBD JPM-TMT reviewed 30 biological agent identifier system submissions to the RFI. The systems represented stages of technology development from advanced concept to COTS instruments. The reviewers separated candidate systems into two categories based on size and technology:

- Handheld – Antibody and Nucleic Acid-based
- Mobile Lab – Antibody and Nucleic Acid-based

The RFI responses were the foundation of this selection, however, other relevant sources of information and systems were also considered (e.g., Emanuel, P. & Caples, M. (2011) The Chemical, Biological, Radiological Technology Survey). Systems were selected to represent a diverse collection of identification methodologies with a focus on unique technology characteristics, stage of development of the systems or the time needed for further development, assay availability, and logistics. After further assessment and characterization, 12 candidate systems were chosen for inclusion in the formal test bed assessment. Those systems and their associated companies are shown in Tables 1 and 2.

Table 1. Selected Nucleic Acid-Based Biological Detection Identifiers

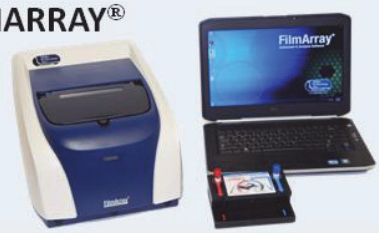
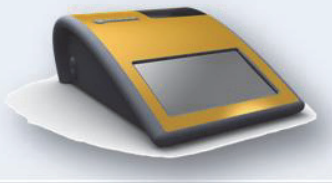
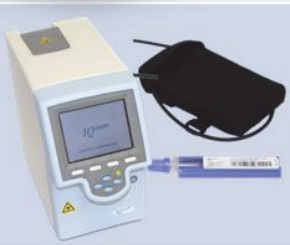
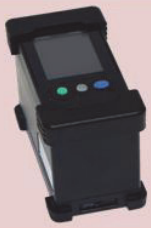
IDENTIFIER	COMPANY	DESCRIPTION
RAZOR® EX BIODETECTION SYSTEM 	BIOFIRE DIAGNOSTICS, INC.	Real-time PCR
FILMARRAY® 	BIOFIRE DIAGNOSTICS, INC.	Nested PCR with melt curve analysis; sample preparation
GENEDRIVE™ SYSTEM 	epistem	PCR with melt curve analysis
PALLADIUM SYSTEM 	INT	PCR and hybridization to complete electrical circuit
LIAT™ ANALYZER 	IQuum	Sample preparation and real-time PCR
T-COR4™ REAL TIME PCR THERMOCYCLER 	Tetracore	Real-time PCR

Table 2. Selected Antibody-Based Biological Detection Identifiers

IDENTIFIER	COMPANY	DESCRIPTION
NIDS® with STAND ALONE READER III 	ANP Tech	Lateral flow immunoassay with reader
MAGPIX® 	Luminex	Fluorescently labeled magnetic microsphere capture antibody
CARTRIDGE READER 	MSD Meso Scale Discovery	Electrochemiluminescence (ECL)
RAPTOR™ BIOASSAY DETECTION SYSTEM 	Research International	Antibody-based wave guide detection
SPINDX™ 	Sandia National Laboratories	Antibody-based capture beads
SPIRIT™ 	Seattle Sensor Systems CORPORATION	Surface plasmon resonance

Selection of System Attributes for Evaluation

The selection panel held several discussions to select relevant information and performance attributes to be captured pertaining to each biological agent identifier system. These attributes were:

- Single Target Identification
- Multiplex Target Identification
- Multiplex Capability
- Assay Flexibility
- Batch Size
- Run Time
- Size
- Power
- Logistical Support
- Costs
- Compatibility and Interchangeability
- Usability
- Maturity

Attribute information was collated into a Biological Identifier Assessment Table (Appendix B). Information was both vendor-supplied and based on the laboratory assessment. The panel scored system attributes based on objective criteria, where possible. Individual attributes for each system were scored on a scale of one to 10 compared to a theoretically ideal (i.e, "perfect ten") technology or product. Each attribute category also received a weight corresponding to its importance and utility. By multiplying the scores and weights, the various biological agent identifiers were ranked in comparison to ideal instruments. The weighting schemes were designed to be scalable so individual end-users would be able to adjust weights for particular attributes based on the end-user's requirements, allowing them to obtain a customized ranking of technologies. In this manner, future acquisitions can be planned by using intelligent decision-making based on flexible and weighted down-selection criteria.

LABORATORY ASSESSMENT OF SYSTEMS

Target Panel

A goal of the laboratory assessment was to directly compare the systems' ability to identify common threats and their versatility to identify various classes of biological threat agents. The specific agents selected to represent these classes include:

- *Bacillus anthracis* (Gram+ spore forming bacilli)
- *Yersinia pestis* (Gram– rod-shaped bacterium)
- Vaccinia (VAC; double-stranded deoxyribonucleic acid (dsDNA) Orthopox virus, Smallpox [Variola] stimulant)
- Venezuelan Equine Encephalitis (VEE; +sense single-stranded RNA virus, an Alphavirus)
- *Clostridium botulinum* Type A neurotoxin (BoNT A; protein toxin)

Assay Development

Of the 12 systems that were down-selected for further assessment, eight did not have assays available for some of the five of the chosen targets in the Target Panel. Therefore, ECBC provided support for assay development to the manufacturers of the following devices:

1. Epistem Genedrive
2. INT Palladium
3. IQuum Liat
4. MesoScale Diagnostics (MSD) Cartridge Reader
5. Research International RAPTOR
6. Sandia National Laboratories SpinDx
7. Seattle Sensors SPIRIT
8. Luminex MAGPIX

Where necessary to develop specific assays, antibodies and inactivated agents were provided to the companies by the Critical Reagents Program (CRP), a component of JPEO-CBD's JPM-MCS. Among other duties, JPM-MCS is responsible for research, development and acquisition of U.S. Food and Drug Administration (FDA)-approved medical systems for diagnostic capabilities against biological threat agents.

Nucleic acid-based biological agent identifiers do not have the capability to directly identify toxins since they are protein-based agents. However, the manufacturers of the Genedrive, Liat, and Palladium attempted to develop immuno-PCR assays. While none of these companies successfully developed immuno-PCR assays for their systems, Epistem, with a commercial partner, succeeded in developing an activity-based BoNT A assay for use on the Genedrive platform. This assay utilizes a synthetic fluorometric substrate to directly measure the presence of *C. botulinum* Type A enzyme proteolytic activity.

The FilmArray, RAZOR EX, and T-COR 4 systems were not able to directly identify toxins and did not attempt to develop assays specific for BoNT A. However, assays were available for the instruments to directly detect DNA from *Clostridium botulinum*, the bacteria that produce the toxin. Native toxin preparations may contain residual DNA from the source organism; therefore, the FilmArray, RAZOR EX and T-COR 4 were also evaluated for the capability to indirectly detect toxin by detecting the *C. botulinum* bacterial DNA.

The FilmArray, RAZOR EX, and T-COR 4 systems had assay kits that were well established and did not need development. The FilmArray had assays for the 4 non-toxin targets while the RAZOR EX and T-COR 4 had assays for *Bacillus anthracis* and *Yersinia pestis*, but not VEE or VAC. A complete list of assays available for each instrument is shown in Table 4.

Three of the antibody-based biological agent identifiers can detect all five targets in multiplex assays and, therefore, assay development was not necessary. The exceptions were the RAPTOR, which did not have a VEE assay, and the SPIRIT, which could not perform multiplex detection using the current analysis software package. Manufacturers of the RAPTOR, SPIRIT, Cartridge Reader, and SpinDx were provided antibodies from the CRP to develop assays. The NIDS has assays for all five targets, but they are distributed across two 5-Plex assay cassettes. Singleplex and multiplex assays for the Luminex MAGPIX were developed by ECBC BioSensors staff using CRP antibodies. The MAGPIX is an analyte capture/multiplex bead based detection system that has the capability of capturing both immunological and molecular events; this study evaluated only the immuno capability.

Pre-Laboratory Assessment of System Specifications and Logistics

The systems underwent an assessment prior to the laboratory evaluation. System Evaluation Worksheets (Appendix C) were completed for each system and contain details on:

- equipment specifications
- shipment of equipment
- shipment of reagents
- set up of equipment
- storage
- shelf life

Laboratory Assessment of System Performance

Biological Agents, Nucleic Acids, and Antibodies

The following inactivated (gamma irradiated) bacterial and viral strains were purchased by ECBC through the CRP and used in the laboratory assessments:

- *Bacillus anthracis* Ames
- *Yersinia pestis* CO92
- VAC Elstree (Lister)
- VEE virus, vaccine strain TC-83

These inactivated bacteria and viruses served as the test agents for the particular assays of interest in antibody-based bio-identifiers. Nucleic acids were derived from the inactivated bacteria and viruses either through on-board or external sample preparation. This study used gamma irradiated agents rather than live cells to minimize safety risks during the completion of mobile laboratory and outdoor field-simulated exercises. Specifications for each strain can be found in Table 3.

Table 3. Specifications of Bacterial and Viral Agents Used in the Laboratory Assessments

Agent	Concentration (Colony Forming Units (CFU)/milliliter (mL) or Plaque Forming Units (PFU)/mL)	Genomic Equivalents (GE)[1]	GE/CFU Ratio	Estimated Genome Size (Kb)[2,3]	GE/nanogram (ng) Nucleic Acid[4]
B. anthracis Ames	6.68×10^{8}	1.59×10^{9}	2.38	5227	1.77×10^{5}
Y. pestis CO92	3.01×10^{9}	4.17×10^{9}	1.39	4830	1.92×10^{5}
VAC Elstree (Lister)	1.31×10^{9}	1.89×10^{9}	1.44	189	4.90×10^{6}
VEE virus, TC-83	1.00×10^{10}	1×10^{10}	1.00	11.4	1.62×10^{8}

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

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

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

[4] http://www.endmemo.com/bio/dnacopynum.php

Antibodies used in this study were purchased by ECBC through the CRP and used in the laboratory assessment. These matched pairs have previously been effectively used in sandwich-type immunoassays. The Seattle Sensors SPIRIT and the Research International RAPTOR systems only required one antibody per each target. The antibodies purchased for testing were:

- 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)
- anti-BoNT A monoclonal antibody (Cat Num: AB-BOT-A-MAB2 Lot Num: 260607-01)

Toxin Source

BoNT A was purchased from Metabiologics, Inc. (Madison, WI) as the active holotoxin complex. The concentration of the toxin was 1mg/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.

Sample Preparation

The systems processed samples from one of these sources:

- whole inactivated agent (all antibody-based systems)
- inactivated agent processed on-board the instrument (FilmArray, Liat) prior to analysis, or by an affiliated sample dilution (RAZOR EX)
- purified DNA via Qiagen DNeasy (T-COR 4, Genedrive)

The basic test sample was comprised of the agent in pristine buffer. Several systems directly analyzed this type of test sample (FilmArray, Liat, NIDS, Cartridge Reader, SPIRIT, SpinDx, MAGPIX, and Raptor). However, RAZOR EX utilized a simple sample dilution step prior to loading on the system, while the T-COR 4 and Genedrive systems required sample preparation in the form of external nucleic acid purification.

Singleplex Assays

Each of the biological agent identifiers was assessed for their ability to identify each of five threat agents prepared in separate test samples. Some manufacturers developed custom assays for inclusion in this assessment. However, most of the nucleic acid platforms were unable to directly detect BoNT A and other systems lacked assays for one or more targets (Table 4).

Table 4. Singleplex Assay Study Design Used in the Laboratory Assessment

SYSTEM		ASSAY AVAILABLE FOR TEST AGENT				
		B. anthracis	*Y. pestis*	VAC	VEE	BoNT A toxin
Nucleic acid-based	FilmArray	✓	✓	✓	✓	✓[1]
	Genedrive	✓	✓	✓	✓	✓[2]
	Liat	✓	✓	✓	✓	-
	Palladium[3]	-	-	-	-	-
	RAZOR EX	✓	✓	-	-	✓[1]
	T-COR 4	✓	✓	-	-	✓[1]
Antibody-based	Cartridge Reader	✓	✓	✓	✓	✓
	MAGPIX	✓	✓	✓	✓	✓
	NIDS SAR III	✓	✓	✓	✓	✓
	RAPTOR	✓	✓	✓	-	✓
	SpinDx	✓	✓	✓	✓	✓
	SPIRIT	✓	✓	✓	✓	✓

[1] The system has an assay for *C. botulinum*, the bacterial source of BoNT A toxin, and was tested for the ability to detect residual host DNA in the BoNT A preparation.

[2] Epistem developed an enzyme activity-based assay for BoNT A.

[3] The Palladium device did not arrive in time to be included in this evaluation.

The vendor-supplied expected limit of detection (LOD) was the basis for a target concentration. The flowchart in Figure 6 demonstrates how vendor LOD claims were evaluated during the laboratory assessment.

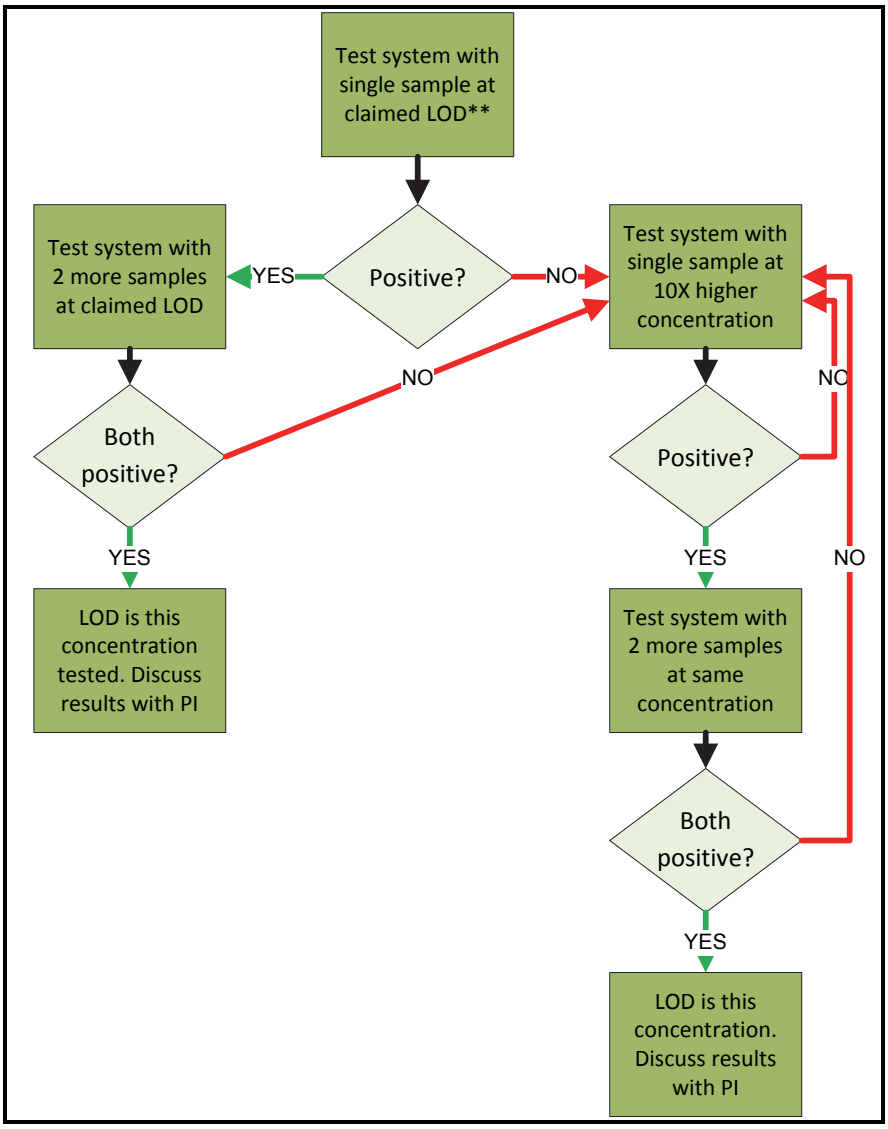


Figure 6. Assessment of Biological Identifier LOD Claims.
****If the LOD was not provided by the vendor testing began at a concentration appropriate for the detection method.**

Information gathered during the singleplex and multiplex testing in ECBC's stationary laboratory was recorded in a System Analysis Report (Appendix C) and added to the Biological Identifier Assessment Tables (Appendix B).

Multiplex Assays

The biological agent identifiers were assessed for their ability to simultaneously identify up to five threat agents in test samples containing all five agents (Table 5). Of the 12 instruments, only four had the capability to test for all five agents. The nucleic acid-based instruments could not detect BoNT A because it was a protein, while some instruments did not have assays available for one or more of the targets. The empirically determined LOD from the singleplex assessment was the basis for a target concentration. When a biological agent identifier failed to detect a threat agent in the multiplex format, additional test samples were prepared at concentrations ten times above this target. Positive samples were verified by triplicate runs.

Table 5. Multiplex Assay Study Design Used in the Laboratory Assessment

SYSTEM		TEST AGENTS
Nucleic acid-based	Film Array	*B. anthracis*, *Y. pestis,* VAC, VEE
	Genedrive	*B. anthracis, Y. pestis,* VAC, VEE
	Liat[1]	*B. anthracis*, *Y. pestis,* VAC, VEE
	Palladium	No Assays
	RAZOR EX	*B. anthracis*, *Y. pestis*
	T-COR 4	N/A
Antibody-based	Cartridge Reader	*B. anthracis*, *Y. pestis,* VAC, VEE, BoNT A
	MAGPIX[3]	*B. anthracis*, *Y. pestis,* VAC, VEE, BoNT A
	NIDS SAR III[1]	*B. anthracis, Y. pestis,* VAC, VEE, BoNT A
	SpinDx	*B. anthracis, Y. pesti,* VAC, VEE, BoNT A
	SPIRIT[2]	N/A
	RAPTOR	*B. anthracis*, *Y. pestis,* VAC, BoNT A

[1] The Liat has assays for four targets on two assays while the NIDS has assays for all five of the targets on two 5-plex cartridges. One cartridge contains 3 of the targets: *Y. pestis*, VAC and VEE and the other contains 2 of the targets: *B. anthracis* and BoNT A toxin.

[2] The SPIRIT is functionally capable of performing multiplex detection of up to three targets but the current analysis software can only determine presence of target in 1 of the 3 available positions.

[3] The MAGPIX multiplex detection assay did not undergo optimization

TEST BED LABORATORY ASSESSMENT SUMMARIES

FilmArray®

by BioFire™ Diagnostics

System Specifications

Vendor: BioFire Diagnostics, Inc.

Website: www.biofiredx.com

System Cost: $49,500.00

Assay Cost: $185

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: 17 (BioThreat Panel) including multiple targets per agent

Assay Shelf Life: 4-6 months at room temperature

Sample Size Required: 250 µL

Type of Detection: Nucleic acid amplification with end point melt-curve analysis

Time to Result: 65 minutes

System Weight: 20 lbs (systems requires laptop and pouch preparation station not included in weight)

Operating Range: 59 - 86 °F (15 - 30 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab.

System Description

The BioFire FilmArray system is a multiplex PCR system that integrates sample preparation, amplification, detection and analysis. The BioThreat Panel v2.4 pouch stores all the necessary reagents for sample preparation, RT-PCR, PCR and detection in a freeze-dried format. Once an unprocessed sample is injected in to the pouch the FilmArray will extract and purify nucleic acids; perform an initial, large volume multiplex PCR; and complete individual singleplex, second-stage PCR reactions to identify specific targets. Finally the system uses meta analysis of endpoint melt curve data to generate a result for each agent based on the results of one or more targets, each performed in triplicate. Each assay contains internal standards to automatically control for each step of the process. The design of this system requires minimal user training and very little hands on time.

Test Bed Review

The FilmArrays' ability to screen 17 different pathogens (25 targets total) in 60 minutes with sensitivity as low as $5x10^2$ CFU/ml makes this system an asset in any laboratory. Although the FilmArray is not "portable", the unique BioThreat pouch had the ability, during our study, to simultaneously detect 3 targets of *Bacillus anthracis*, 2 targets of *Yersinia pestis*, 2 targets of VEE virus and 2 targets of Orthopox virus. During testing the targets were run as singleplex samples, and then the system was challenged using multiple targets in a single sample. The FilmArray was able to detect all 4 targets with no loss to sensitivity.

During the mobile laboratory, scientists were impressed with the ease of use that the pouch and its associated stand offered. A quick 10 minute training with each end-user allowed them to run the system independently with one scientist commenting, "I wish every system was this easy to run". The biggest impact the system had on the Mobile Lab scientists was its multiplex capability. "For screening an unknown sample, this system would be invaluable", one commented. With time not being an immediate concern for this group, the FilmArray was among their top picks of systems.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	$5.00x10^{2}$	⚠	$5.00x10^{3}$
Yersinia pestis	$5.00x10^{1}$	✓	$5.00x10^{0}$
Vaccinia	$1.00x10^{2}$	⚠	$1.00x10^{3}$
Venezuelan Equine Encephalitis	$1.00x10^{3}$	✗	$1.00x10^{6}$
Clostridium Botulinum Toxin	N/A	○	N/A
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$5.00x10^{3}$	✓	$5.00x10^{3}$
Yersinia pestis	$5.00x10^{0}$	⚠	$5.00x10^{1}$
Vaccinia	$1.00x10^{3}$	✓	$1.00x10^{3}$
Venezuelan Equine Encephalitis	$1.00x10^{6}$	✓	$1.00x10^{6}$
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated ⚠ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

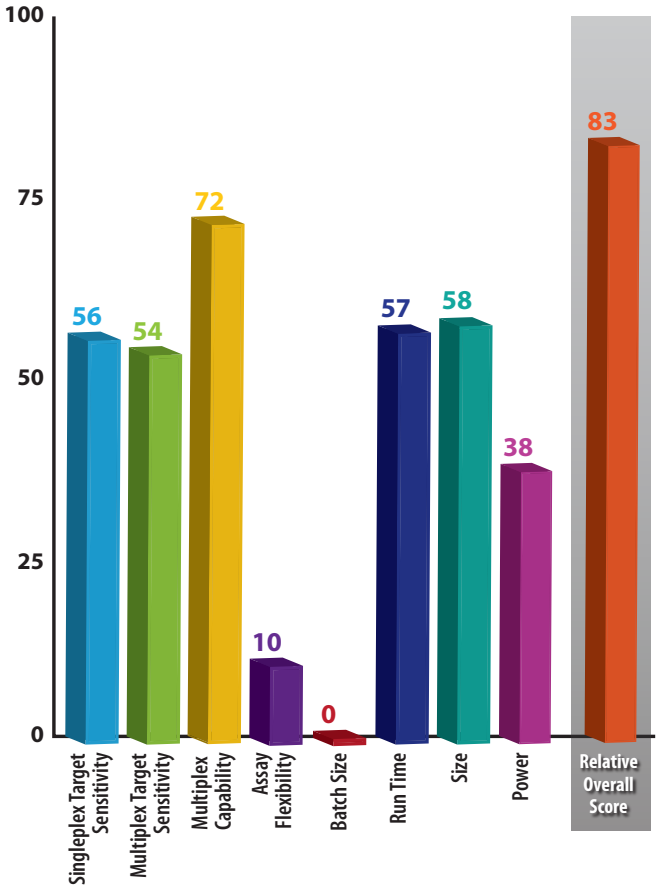
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

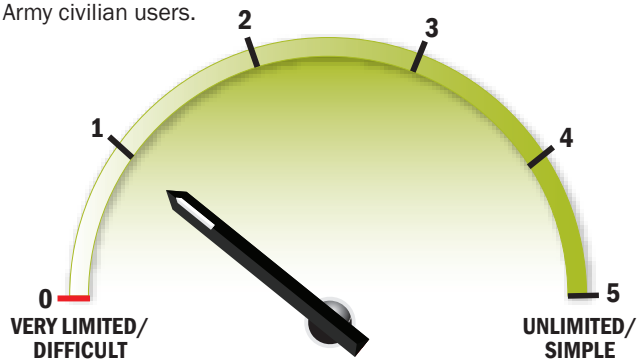
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



RAZOR®

by BioFire Diagnostics, Inc.

System Specifications

Vendor: BioFire Diagnostics, Inc.

Website: www.biofiredx.com

System Cost: $38,500.00

Assay Cost: $200.00

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: 10

Assay Shelf Life: 1 year at room temperature

Sample Size Required: 250 µL

Type of Detection: Nucleic Acid

Time to Result: 25 minutes

System Weight: 11 lbs

Operating Range: 32–104 °F (0–40 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab and field.

1 2 3 4 5 6 **7** 8 9

System Description

BioFire's RAZOR EX is a field PCR unit that uses pouches pre-loaded with freeze-dried PCR reagents for the detection and identification of biological pathogens and biothreat agents. Each kit contains all of the items necessary for sampling, sample preparation and real-time PCR. Each kit includes items needed for collecting and loading the sample. Once samples are loaded into the pouch with cannula-tipped syringes they are dispensed automatically in to the wells, requiring no precise measuring. BioFire has assays available for CDC defined Category A and B Biothreat pathogens. One pouch will test for 3 to 10 different agents, depending on configuration, and includes internal controls to validate the integrity of the test. BioFire's pouches and its associated kit components are manufactured under a cGMP quality system. This testing used The TEN™ 10 Target Screen Kit to detect 2 out of 4 desired targets, *Bacillus anthracis* and *Yersinia pestis*, and verify vendor claims of limits of detection (LOD). Additionally, the Botulinum toxin preparation was tested for residual C. botulinum DNA.

Test Bed Review

The RAZOR was one of the few systems in this test that had been designed as a field-ready system. Because of this, the expectation that it would outperform the others was there, however the ease of use did not meet up with the end users requirements.

In the laboratory the system performed comparable to the other PCR systems, with LOD's down to 102, for its *Yersinia pestis* assay. Unfortunately, BioFire's RAZOR is considered a "closed" system, the company was not willing to develop new assays for this study therefore only 2 (*Yersinia pestis* and *Bacillus anthracis*) of the desired 5 targets were tested. This closed system is an unfavorable characteristic for military applications that are challenged with new threats and require new assays at any given time.

During the field testing, soldiers had difficulty manipulating the pouch and found the barcode scanner difficult to use. One commented, "This system is tedious". Another concern for the soldiers in the field was the difficulty they had reading the screen in the daylight. One soldier noted "the brightness option does not help in the bright sunlight". Overall the field ready system isn't as ready as it appears. In full MOPP gear the consumables gave the greatest challenge even for the simplest of tasks, such as opening a box.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	$1.00x10^3$	⚠	$1.30x10^4$
Yersinia pestis	$1.00x10^2$	⚠	$1.30x10^3$
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$1.30x10^4$	✓	$1.30x10^4$
Yersinia pestis	$1.30x10^3$	✓	$1.30x10^3$
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated ⚠ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

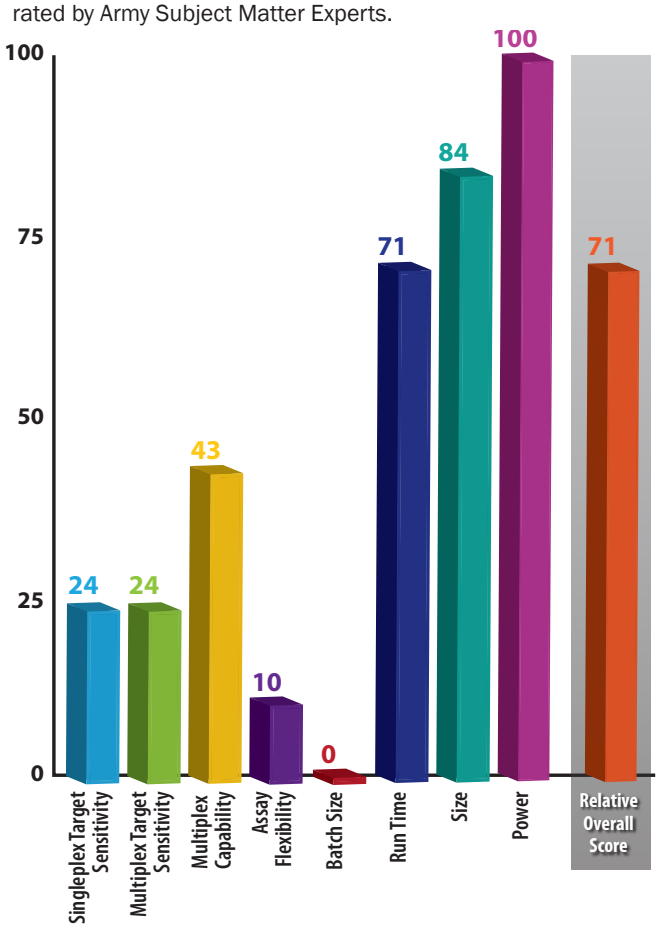
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

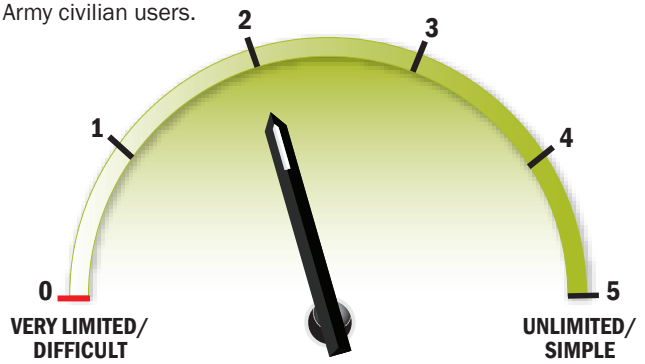
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Genedrive™

by Epistem, Inc.

System Specifications

Vendor: Epistem, Inc.

Website: www.epistem.co.uk

System Cost: $4,000

Assay Cost: Price Request (Price estimated to be $85/assay, subject to quantity)

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: 4 per Assay

Assay Shelf Life: Unknown

Sample Size Required: 20 µL

Type of Detection: Nucleic Acid

Time to Result: 60 minutes

System Weight: 1.2 lbs

Operating Range: 32–131 °F (0–55 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System is still in prototype stage. Some components need refinement.

System Description

The Genedrive is a fully integrated, endpoint PCR-based platform. Genedrive's proprietary "hybrid thermal engine" allows faster cooling rates and shorter annealing times relative to conventional PCR devices. The system is capable of performing ultra fast PCR cycling of 30 cycles in as little as 17 minutes and is controlled by a single button. The Genedrive was designed to be a highly cost-effective way of moving molecular diagnostics from the laboratory to the point of need across several markets including government. Epistem currently has a *Mycobacterium tuberculosis* IVD assay for the Genedrive that has received European approval. For this testing Epistem developed multiplex assays to detect *Bacillus anthracis*, *Yersinia pestis*, Vaccinia and Venezuelan Equine Encephalitis and a singleplex assay to detect active Botulinum neurotoxin type A.

Test Bed Review

On paper, the Genedrive's low cost, small footprint and short run time were appealing; unfortunately, the assays did not meet expectations and required modified testing procedures to achieve successful data. After several iterations, including eliminating the sample preparation paper and making internal hardware structure changes, the system was able to detect approximately 1.66×10^5 CFU/mL in the *Yersinia pestis* assay. However, *Bacillus anthracis* and *Vaccinia* detection limits were 100 fold higher and the device couldn't run the VEE assay because of firmware shortcomings. The 60 minute run time was unexpected, since the system was projected to perform 30 cycles in 17 minutes. Genedrive also produced false positives and inconsistent results. Our testing demonstrated that unrefined sample preparation and assay reagents also contributed to poor performance.

Field-users were impressed with the size of the system, but little else. Surprisingly, the sample preparation created the hardest task of manipulating several tubes. One user commented, "Anything that requires several steps would require two operators for set up and therefore would not be used in the field". One soldier noted that the humid weather prevented the sample preparation cards from completely drying. Another user commented "The device needs some method of data accessibility and storage such as a thumb drive for chain of evidence."

Genedrive did not perform well for bacterial and virus detection but was the only PCR-based system that could detect the presence of toxin. The system design is promising and grabs one's attention, but assay design and sample preparation require additional development, putting this system near the bottom for performance in our testing.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = GE, Virus = GE, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	10	✗	69,000
Yersinia pestis	10	✗	200
Vaccinia	10	✗	34,000
Venezuelan Equine Encephalitis	10	○	Not Tested
Clostridium Botulinum Toxin	No Claim	○	10,000
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	69,000	○	N/A
Yersinia pestis	200	○	N/A
Vaccinia	34,000	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	10,000	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

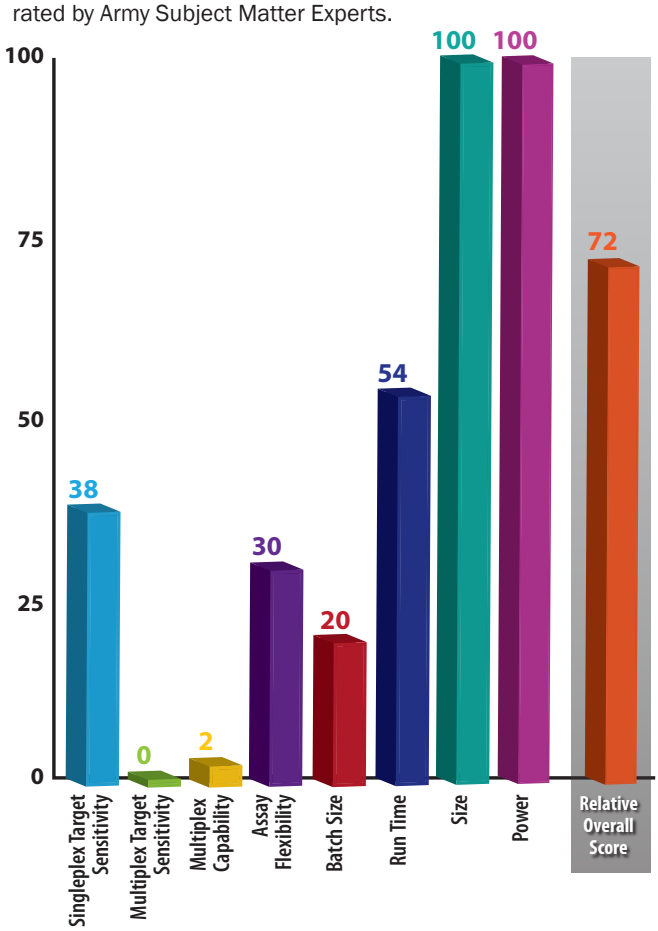
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

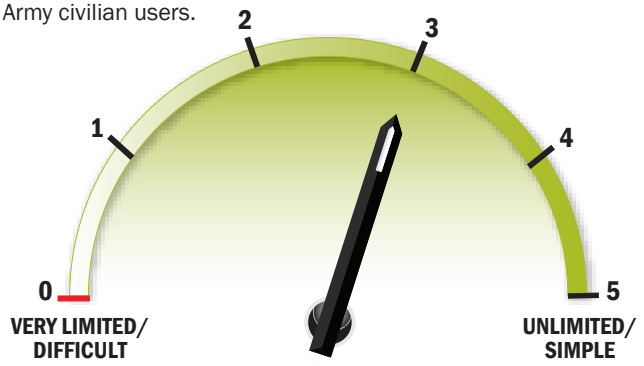
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Liat™

by IQuum

System Description

The Liat is an automated sample-to-result detection analyzer. IQuum's lab-in-a-tube (Liat) was designed to enable non-specialized personnel to perform "moderate complexity" tests in hospital labs or other near-patient setting. The Liat has automated sample processing in a flexible tube containing pre-packaged reagents. Peristaltic manipulations by actuators in the analyzer move the sample through each stage of sample processing ending with a PCR or RT-PCR amplification the sample for target identification. Currently IQuum has a FDA 510(K) cleared Liat Influenza A/B assay on the market which is intended for use in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA). For this testing IQuum developed two duplex assays, one that detects *Bacillus anthracis* and *Yersinia pestis* and another to detect Vaccinia and Venezuelan Equine Encephalitis.

System Specifications

Vendor: IQuum

Website: www.iquum.com

System Cost: $25,000.00

Assay Cost: Price on Request (Estimated to be $60 per test)

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: 2

Assay Shelf Life: 1 year at 4 °C

Sample Size Required: 200 µL

Type of Detection: Real-time nucleic acid amplification and fluorescence detection

Time to Result: 30 minutes

System Weight: 8.3 lbs

Operating Range: 40 - 122 °F (4 - 50 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab and field.

Test Bed Review

A combination of small footprint, automated sample prep, 20 minute run and sensitivity down to the single digits (CFU/mL), the Liat analyzer was a top performer both in the lab and in the field. Achieving detection levels down to 6.5 CFU/mL in their *Yersinia pestis* duplex assay, the Liat's sensitivity was superior to most other PCR platforms in the test bed. In addition to performing well in the presence of one agent, IQuum also scored well in the multiplex category, showing little to no loss of signal when samples were combined in a duplex format. During testing, the Liat did present with several error messages that required IQuum's intervention. However, these errors were a result of system checks put in place as a requirement for a FDA approved system.

In the field, the end-users were impressed with the ease of set-up and minimum amount of training required. One operator stated that "This device is the easiest piece of equipment I've ever used." Admittedly the current configuration of this system was not intended for outdoor use and end-users would like to see some modifications to its current design. The size of the buttons and the use of the stylus were top on their list. One user commented "The Login/Pin requirement is overkill for military applications and the stylus would get lost".

As a result of testing in an analytical and mobile laboratory and the field, the Liat showed a great deal of potential. We believe with little investment in its design (i.e., ruggedization of the exterior, integrated battery) this system would be a good fit in most, if not all, military testing applications.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX

Agent	Vendor Claimed LOD	Validation of Claimed LOD	Actual LOD
Bacillus anthracis	$2.00x10^{1}$	✗	$1.00x10^{3}$
Yersinia pestis	$5.00x10^{0}$	✓	$6.5x10^{0}$
Vaccinia	$2.00x10^{2}$	△	$2.50x10^{3}$
Venezuelan Equine Encephalitis	$4.00x10^{4}$	✓	$2.10x10^{3}$
Clostridium Botulinum Toxin	N/A	○	N/A

MULTIPLEX

Agent	Actual Singleplex LOD	Actual LOD Achieved	Multiplex LOD
Bacillus antracis	$1.00x10^{3}$	✓	$1.00x10^{3}$
Yersinia pestis	$5.00x10^{0}$	△	$1.00x10^{1}$
Vaccinia	$2.50x10^{3}$	△	$2.50x10^{4}$
Venezuelan Equine Encephalitis	$2.10x10^{3}$	△	$2.10x10^{4}$
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

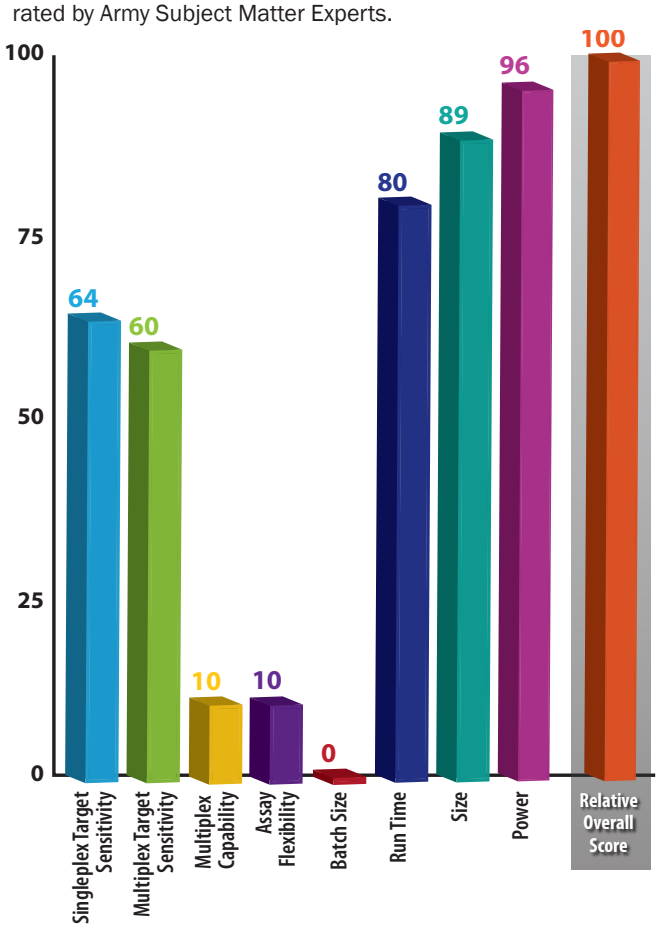
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

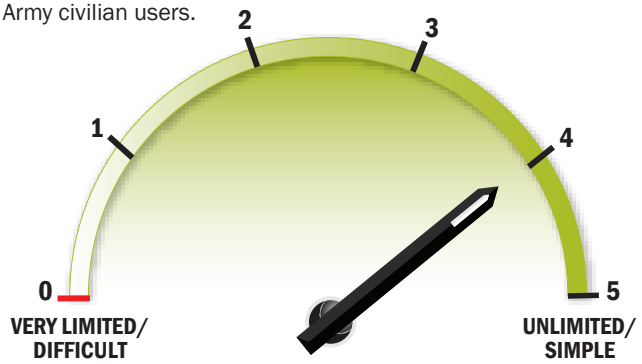
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



T-COR 4™

by Tetracore, Inc.

System Specifications

Vendor: Tetracore, Inc.

Website: www.tetracore.com

System Cost: $38,500.00

Assay Cost: $16,000.00

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: One. Multiple formats available

Assay Shelf Life: 1 year at room temperature

Sample Size Required: 3 µL

Type of Detection: Nucleic Acid

Time to Result: 45 minutes

System Weight: 6.2 lbs (w/o required centrifuge)

Operating Range: 39–113 °F (4–50 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab and field.

System Description

The T-COR 4 is a portable Real-Time PCR thermocycler with four independent sample wells capable of 2 color detection. The T-COR 4 is a field deployable battery powered system, but lacks sample preparation capabilities. Tetracore's real-time PCR reagents are stored at room temperature with 20+ assays currently available. The system in encased in a heavy duty protective rubber sleeve with an internal 8 hour battery for the thermal cycler. For this testing, Tetracore's *Bacillus anthracis* pXO1 assay and *Yersinia pestis* assay were evaluated. Each assay contains reagents for specific target detection using the FAM fluorophore and an internal control detected with CY5 fluorophore.

Test Bed Review

The T-COR 4 is small, lightweight and battery operated, making it appealing for the field. The main shortcomings were the number of targets (1) per test and lack of integrated sample preparation. With purified samples, the system scores high in the laboratory, sensitivities in the femtogram range. The 20 minute runtime and real time viewing are appealing, unfortunately without on board sample preparation, the scores are lower than the other systems. Additionally, Tetracore was only able to provide 2 of the 5 requested targets, with no ability to design new assays for this testing. The test results could not be saved on the device, as configured. Tetracore can provide software to operate the instrument, and save and analyze data using an external computer.

In the field, the end-users liked the ease-of-use of the system and had no trouble running this system independently. Although one soldier commented, "It doesn't really add anything additional", another added, "It's quick to start up and easy to use". End users liked that the results were easy to view and interpret. Consistent results were an additional plus of this system. Training time was minimal and the consumables were easy to handle in MOPP gear. The small centrifuge for the PCR tubes added to the footprint for field operators.

As a result of testing in an analytical and mobile laboratory and the field the T-COR 4 was a consistent and easy to use system. The lack of onboard sample preparation is a negative, but the system is a reliable workhorse.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = fg/uL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	No Claim	○	$3.2x10^{-1}$
Yersinia pestis	No Claim	○	$1.3x10^{1}$
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus anthracis	$3.2x10^{-1}$	○	N/A
Yersinia pestis	$1.3x10^{1}$	○	N/A
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated ⚠ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

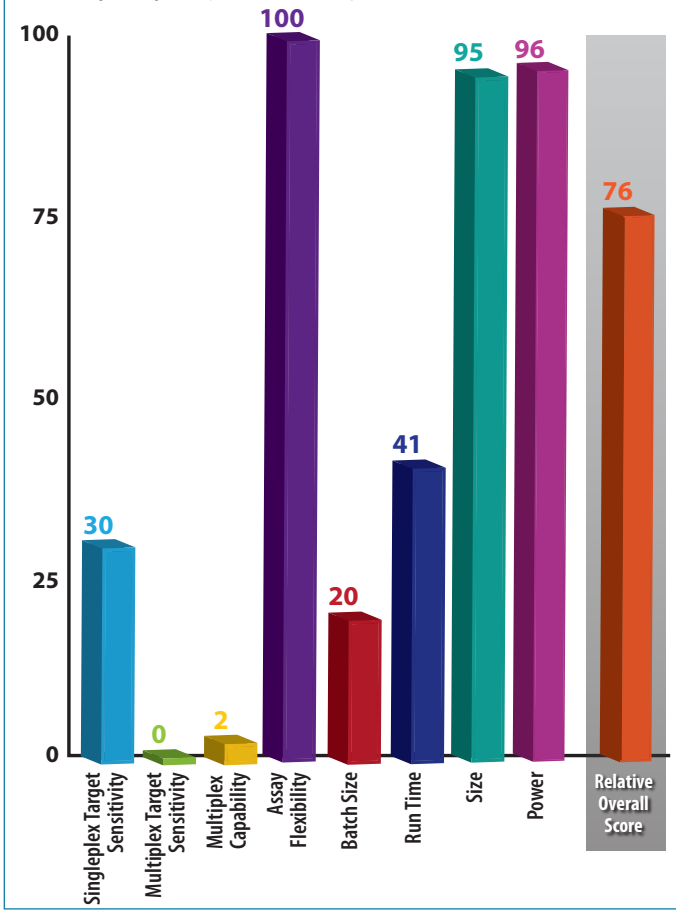
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

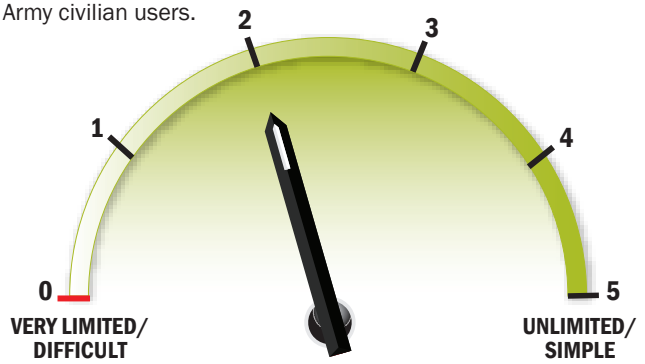
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



NIDS®

by ANP Technologies

System Specifications

Vendor: ANP Technologies

Website: www.anptinc.com

System Cost: $6,500.00

Assay Cost: $45.00

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: 5 per Assay, Multiple formats available

Assay Shelf Life: 2 years from receipt at room temperature

Sample Size Required: 100–200 µL

Type of Detection: Antibody

Time to Result: 15 minutes

System Weight: 1.6 lbs

Operating Range: 40–122 °F (4–50 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). NIDS assay used with Stand Alone Reader had successful operation in Mobile lab and field.

System Description

ANP has developed the Nano Intelligent Detection System, or NIDS. The NIDS is a multiplexed Handheld Assay (HHA) together with a palm-sized, portable, ruggedized optical Stand Alone Reader (SAR III). The NIDS technology uses an antibody nanomanipulation technique that orients each antibody so that there are optimal biosensing regions available for antigen binding and sandwich formation. Because of this nanomanipulation, the NIDS is the first HHA that claims to have no "Hook Effect". The NIDS was designed to take all of the guess work out of interpreting HHA results by different responders in field conditions with poor lighting and limited visibility while wearing personal protective equipment.

Test Bed Review

The NIDS is small, rapid and has the ability to detect bacterial, viral, and protein toxins. However, it still delivers below average sensitivity and high false positive rates that we've come to expect with a typical HHA. Detection levels ranging from $1x10^6$ to $1x10^8$ CFU/mL for a singleplex assay and $1x10^6$ to $1x10^9$ CFU/mL in the multiplex, gave the NIDS an overall low score in the Target Identification Category of the Criteria Table.

Where the NIDS did fair well is ease of use and small size. Little to no sample preparation time is required for this system, maintenance time is low and the overall sample to result is 15 minutes. These attributes yielded scores of 70-100% in 4 of the 5 categories.

In the field, the end-users were impressed with the ease of set-up and minimum amount of training required. However one operator, familiar with typical HHAs remarked "The reader makes it easier than current HHAs, but it's still an HHA." In addition, one user experienced false positives while running the device, which was quickly fixed after cleaning the lens. However this result did lead to concerns of contamination. On a positive note, one soldier commented, "The device is small enough to fit into the pouch of my body armor".

As a result of testing in an analytical and mobile laboratory and in the ECBC Skippers Point Site the NIDS overall performance was mediocre at best. As one of the only currently fielded systems in our test bed, better results were expected from this system and users were disappointed by the recurring false positives. Clearly there is a need for a more sensitive and accurate identification of biothreats

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	$1.00x10^{6}$	⚠	$1.00x10^{7}$
Yersinia pestis	$2.50x10^{5}$	⚠	$2.50x10^{6}$
Vaccinia	$1.00x10^{6}$	✖	$>1.00x10^{8}$
Venezuelan Equine Encephalitis	$1.00x10^{8}$	⚠	$1.00x10^{9}$
Clostridium Botulinum Toxin	$5.00x10^{1}$	✔	$5.00x10^{1}$
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$1.00x10^{7}$	✔	$1.00x10^{7}$
Yersinia pestis	$1.00x10^{6}$	✔	$1.00x10^{6}$
Vaccinia	$>1.00x10^{8}$	✔	$1.00x10^{8}$
Venezuelan Equine Encephalitis	$1.00x10^{9}$	✔	$1.00x10^{9}$
Clostridium Botulinum Toxin	$5.00x10^{1}$	⚠	$5.00x10^{2}$

✔ Validated ⚠ Not Validated (≤1 log difference) ✖ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

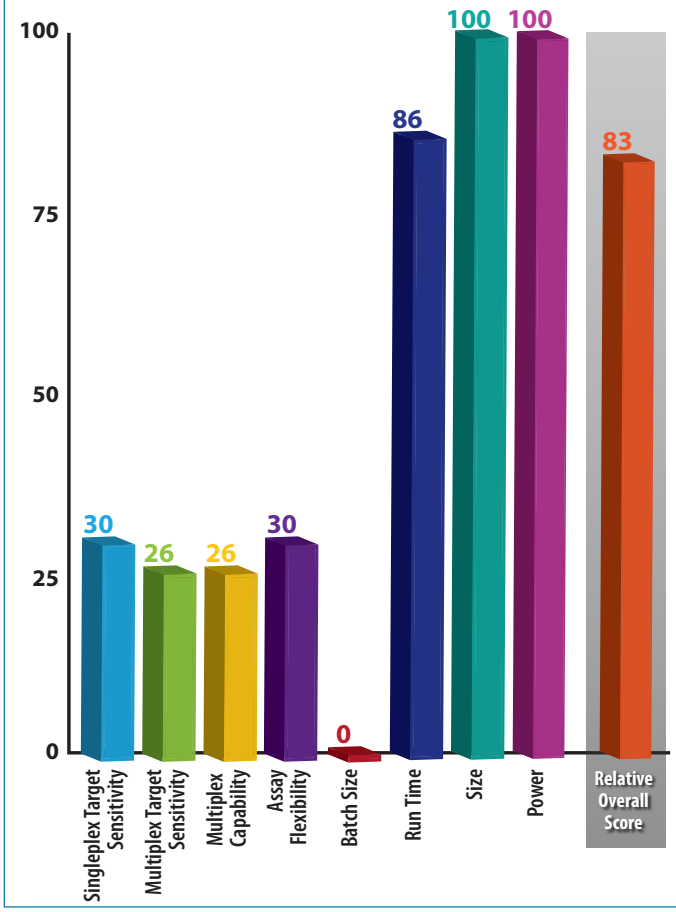
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

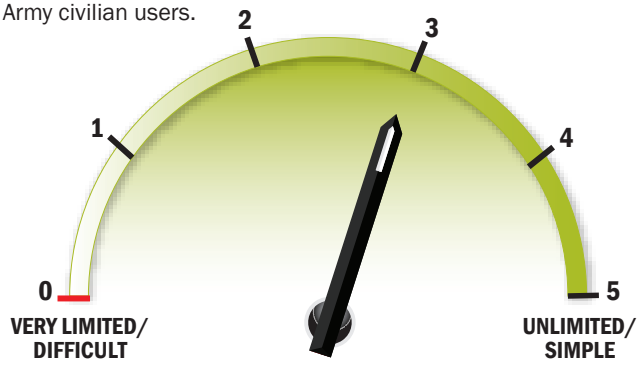
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



MagPix™

by Luminex, Corp

System Specifications

Vendor: Luminex, Corp.

Website: www.luminexcorp.com

System Cost: $24,000.00

Assay Cost: Price on Request

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: Up to 50

Assay Shelf Life: User determined

Sample Size Required: 20–200 µL

Type of Detection: Antibody

Time to Result: 90–105 minutes for No Wash Assay

System Weight: 38.5 lbs

Operating Range: 50–104 °F (10–40 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). Assay integration requires development and optimization.

1 2 3 4 5 **6** 7 8 9

System Description

The Luminex MAGPIX utilizes labeled magnetic beads in a fluidics system combined with optical detection and computerized analysis to perform plate-based multiplex immunoassays. Preparation of the sample(s) is done in a 96-well plate on the benchtop or in a biological safety cabinet and consists of multiple incubations with antibody- and fluorescently-labeled magnetic beads. The operator sets assay parameters in xPONENT software on a computer that controls the MAGPIX and loads the assay plate to the MAGPIX, which runs the analysis of the prepared samples. The MAGPIX can run highly multiplexed assays to measure up to 50 different analytes simultaneously in a sample. The MAGPIX also has capability to perform nucleic acid detection utilizing hybridizations to nucleic acid-labeled magnetic beads and has developed a prototype sample preparation cartridge that would automate sample preparation. For this testing, all assays were developed by the laboratory scientists at ECBC.

Test Bed Review

MAGPIX had one of the largest footprints of the biological identifiers, including computer, magnetic bead separator and a plate shaker. Despite the utilization of a faster "no-wash" ELISA-type assay format, the assays still took approximately 2 hours. The software required training and practice to become comfortable at running assays and analyzing the data. MAGPIX assays utilized the same antibodies as other systems in this assessment, yet were more sensitive than most other devices. Limits of detection were approximately 10^5 CFU/ mL sample for bacteria assays, 10^7 PFU/mL sample for virus assays and 1 ug/mL for toxin assays.

Because of its size, the MAGPIX was assessed in the Mobile Laboratory, but not the field setting. Mobile lab end-users gave mostly split opinions (among FAIR, GOOD and EXCELLENT scores) on the MAGPIX. The exception was for "Ease of Use" which rated FAIR, since this instrument requires some training and more sample preparation than most other biological agent identifiers. One operator with flow cytometry experience was comfortable with the MAGPIX and affirmed, "The MAGPIX is similar to other Luminex devices." The perceived safety was ranked either FAIR or GOOD due to operators concerns over handling the 96-well plates with possible pathogens. Users felt they could minimize the safety concerns by extensive use of bleach and the biological safety cabinet within the Mobile laboratory. The operators suggested, "The MAGPIX should be used as a confirmatory technology, after an initial positive PCR assay."

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	N/A	○	$1.00x10^5$
Yersinia pestis	N/A	○	$1.00x10^5$
Vaccinia	N/A	○	$1.00x10^7$
Venezuelan Equine Encephalitis	N/A	○	$1.00x10^8$
Clostridium Botulinum Toxin	N/A	○	$1.00x10^3$
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$1.00x10^5$	✗	$1.00x10^7$
Yersinia pestis	$1.00x10^5$	△	$1.00x10^6$
Vaccinia	$1.00x10^7$	✗	$>1.00x10^8$
Venezuelan Equine Encephalitis	$1.00x10^8$	✓	$1.00x10^8$
Clostridium Botulinum Toxin	$1.00x10^3$	✓	$1.00x10^3$

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

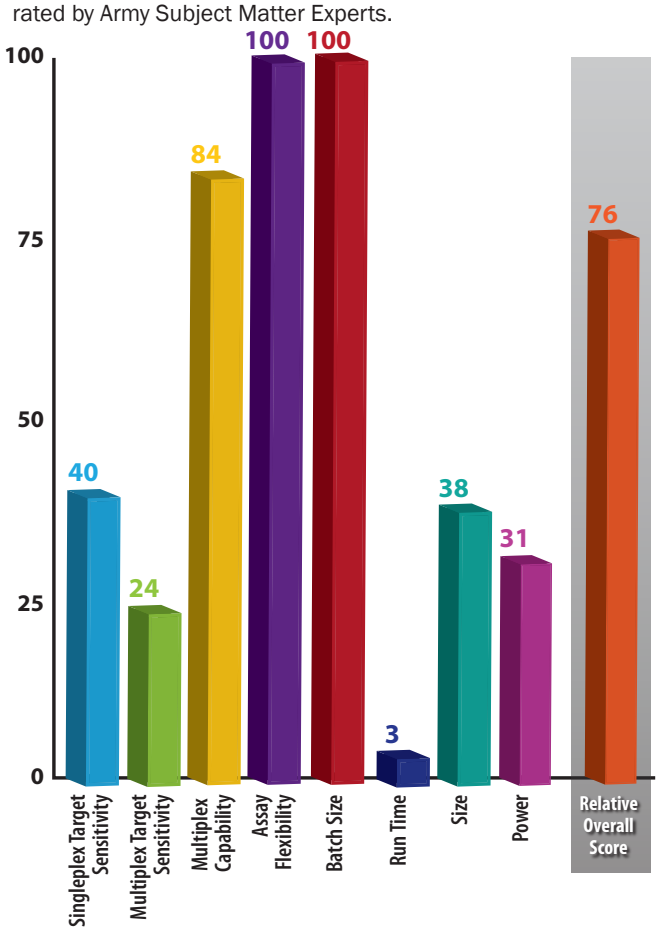
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

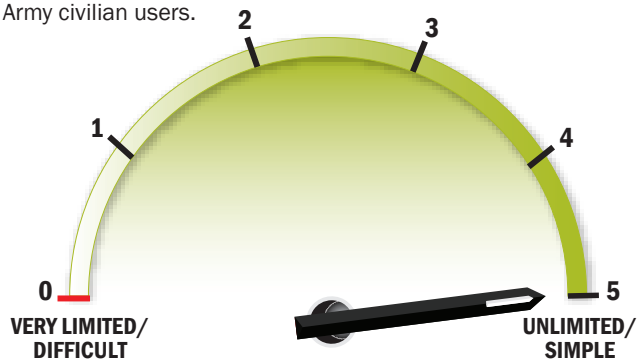
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Cartridge Reader

by Meso Scale Discovery (MSD)

System Specifications

Vendor: Meso Scale Discovery (MSD)

Website: www.meso-scale.com

System Cost: Price on Request

Assay Cost: Price on Request

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: 5

Assay Shelf Life: 1 year at 4 °C

Sample Size Required: 165 µL

Type of Detection: Antibody

Time to Result: 30 minutes

System Weight: 13 lbs

Operating Range: 59–86 °F (15–30 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab and field. Prototype/Beta test unit.

System Description

The Cartridge Reader is one of the few non-COTS systems included in this testing. MesoScale Diagnostics' (MSD) Cartridge Reader uses Multi-Array electrochemiluminescence (ECL) technology to provide highly-sensitive multiplexed detection in a small volume of liquid sample. The instrument carries out measurements using single-use injection-molded fluidic cartridges that can conduct multiplexed measurements of up to 12 targets in a sample. Each cartridge has integrated positive and negative controls. Integrated fluidics on-board the cartridge allow for fully automated sample processing and analysis.

Test Bed Review

As a top performer and end user favorite, the Cartridge Reader was easy to use, featured multiplex capability and consistently performed throughout the entire testing.

Achieving detection levels down to $1x10^5$ cfu/ml in both its *Bacillus anthracis* and *Yersinia pestis* singleplex assays, the Cartridge Readers' sensitivity was comparable to other fully developed antibody-based system. In addition to performing well in their singleplex assay design, MSD also scored well in our "Multiplex" category. This technology was able to detect all five targets with no loss of signal when combined in a duplex format. During the initial laboratory testing, the user interface displayed raw data and required exporting the data to an EXCEL macro file to calculate the final results.

Prior to field testing, MSD was contacted and asked to modify the user interface so that results were easily interpretable by the end user. MSD was able to perform a firmware upgrade and testing within one week. In the field, the end-users were impressed with the ease of set-up and minimum amount of training required. Soldiers referred to the system as "Superior" and a "Favorite" of the antibody based systems. One end-user commented that the system seemed "almost too easy". Where this system lacks the sensitivity of a PCR-based technology it excels in its ability to analyze up to 12 different agents in one sample.

Admittedly the current configuration of this system was not intended for outdoor use because it offers no battery option and the system has not been ruggedized. However these characteristics were minor concerns for the end-users who viewed this system as one of their top picks.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	$1.00x10^{5}$	✓	$1.00x10^{5}$
Yersinia pestis	$1.00x10^{7}$	✓	$1.00x10^{5}$
Vaccinia	$1.00x10^{5}$	✗	$1.00x10^{7}$
Venezuelan Equine Encephalitis	$1.00x10^{8}$	✓	$1.00x10^{8}$
Clostridium Botulinum Toxin	$7.00x10^{-2}$	✗	$1.00x10^{2}$
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$1.00x10^{5}$	✓	$1.00x10^{5}$
Yersinia pestis	$1.00x10^{5}$	✓	$1.00x10^{5}$
Vaccinia	$1.00x10^{7}$	✗	$>1.00x10^{8}$
Venezuelan Equine Encephalitis	$1.00x10^{8}$	✓	$1.00x10^{8}$
Clostridium Botulinum Toxin	$1.00x10^{2}$	△	$1.00x10^{3}$

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

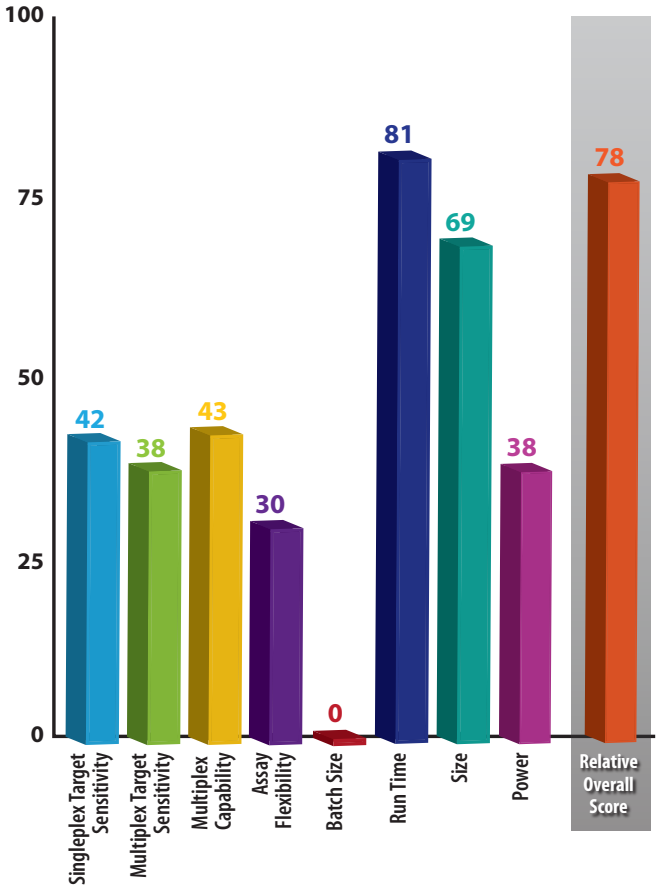
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

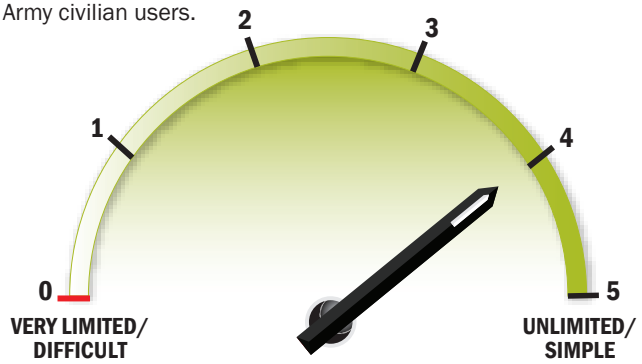
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



RAPTOR™ Plus

by Research International, Inc.

System Specifications

Vendor: Research International, Inc.

Website: www.researchintl.com

System Cost: $49,500.00

Assay Cost: $150

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: 4

Assay Shelf Life: 1 year at 4 °C

Sample Size Required: 1-2 mL

Type of Detection: Antibody

Time to Result: 28 minutes

System Weight: 14.6 lbs (with battery)

Operating Range: 34 - 95 °F (1 - 35 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System completed but had recurring technical issues.

System Description

The RAPTOR by Research International is a field-ready ruggedized fluorometric assay system about the size and weight of a car battery that can be used to detect biological agents, chemical contaminants or explosives. For biological agent identification, the RAPTOR uses a four channel wave-guide system with specific capture antibodies bound to an immunoassay "coupon" for detection and identification of potential threat agents. The operator must prepare and emplace the detector antibody tubes, a pouch with running buffer and a waste pouch prior to operation. The RAPTOR has two internal peristaltic pumps that control the fluids' movements and the assay progression. The total time for set-up, system test and establishment of a pre-operational baseline reading is approximately 30 minutes, while the sample run time is 14 minutes. The instrument is not quantitative; however, detection signals are displayed as *negative, suspect, positive*, and *highly positive* results, providing some indication of relative quantity of a particular target.

Test Bed Review

The laboratory assessment for the RAPTOR utilized its Bioassay Coupon kit, which included assays for *Bacillus anthracis*, *Yersinia pestis*, Vaccinia virus and BoNT A. The technical staff at Research International was not able to develop a working assay for Venezuelan Equine Encephalitis virus. The RAPTOR did not fare well in the laboratory assessment as positive results were not obtained at all for *Bacillus anthracis* or Vaccinia virus at up to 100x the claimed LOD, while *Yersinia pestis* was only positive at 5 x 10^7 CFU/mL, or 10x the claimed LOD. The RAPTOR required some practice to become adept at making the proper fluidics connections prior to operation. The tubing was prone to kink and color coded connectors were mismatched. Also, an interior module that holds the detector antibody tubes had to be frozen prior to operation, possibly causing inconsistent assay conditions as the instrument warmed throughout the day.

The RAPTOR was assessed in the Mobile Laboratory and by both field operator groups. However, 5 of the 8 operators were unable to complete sample analysis because the RAPTOR failed the fluidics pre-operational testing and would not operate correctly, or failed during the sample analysis. Consequently, the operators' rating of "Ease of Use" varied. The manipulation of tubing and pouches prior to sample analysis was difficult for operators in multiple glove layers. One operator reflected, "You shouldn't have to be a mechanic to set-up the device." The RAPTOR received consistently positive ratings for data viewing and interpretation because the surface display is simple to read and understand.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	$5.00x10^4$-$5.00X10^5$	✖	$>1.00x10^7$
Yersinia pestis	No Claim	○	$5.00x10^7$
Vaccinia	$1.00x10^5$	✖	$>1.00x10^7$
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	1-10	✖	$1.00x10^4$
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$>1.00x10^7$	✖	$>5.00x10^6$
Yersinia pestis	$5.00x10^7$	✔	$5.00x10^7$
Vaccinia	$>1.00x10^7$	✖	$>1.00x10^7$
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

✔ Validated △ Not Validated (≤1 log difference) ✖ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

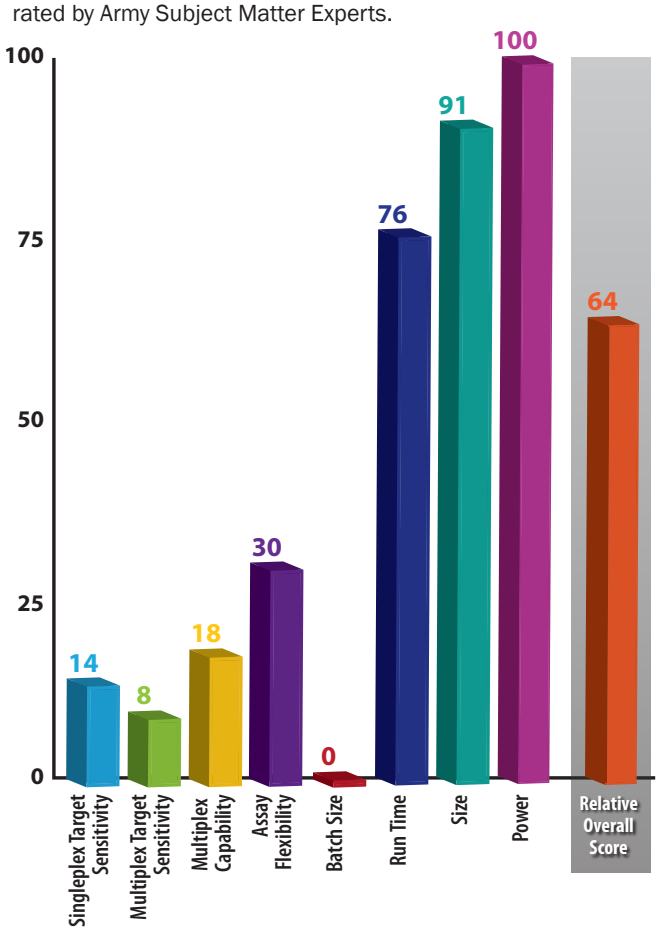
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

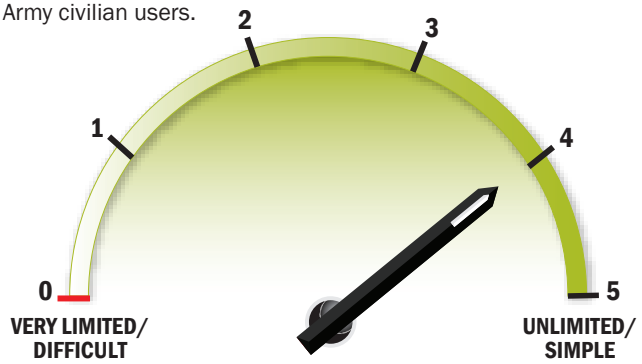
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



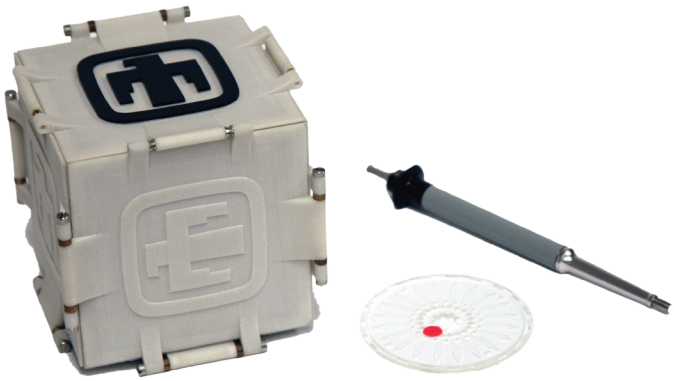
Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



SpinDx™

by Sandia National Laboratory



System Specifications

Vendor: Sandia National Laboratory

Website: N/A

System Cost: To be determined (non-COTS device)

Assay Cost: $2 per disk

Assay Storage Requirements: 4 °C

Agents Tested per Assay: Up to 20 possible

Assay Shelf Life: 6 months at 4 °C

Sample Size Required: 2 µL

Type of Detection: Fluorescent labeled antibodies

Time to Result: 20 minutes

System Weight: 3.5 lbs (system also requires laptop)

Operating Range: 59 - 86 °F (15 - 30 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System is early prototype unit, components are not final. Assay development in progress.

System Description

The SpinDx by Sandia National Laboratory is a non-COTS system designed primarily for rapid diagnostics in a clinical or point-of-care setting. The system utilizes a spinning disk, or "lab on a CD", to draw a sample through a separation matrix while simultaneously binding to fluorescently labeled antibody-bead constructs. The SpinDx uses LED illumination and a photodiode to detect the target. Preliminary results from Sandia show the system to have greater detection sensitivity than standard ELISAs. The SpinDx has also been used to separate whole blood samples for cell counts and other clinical analyses. Operation of the system requires little or no training, and samples require no preparation except for mixing with the analytical matrix. The SpinDx is controlled by a laptop computer via wireless (Bluetooth) communications. With an analysis time of less than 20 minutes, no sample preparation, battery power and small size, the SpinDx has potential to be a prototypical mobile laboratory or even hand-held instrument.

Test Bed Review

The results of the laboratory assessment indicate the SpinDx is still at the developmental prototype stage. Sandia utilized Critical Reagent Program antibodies to create assays for all 5 targets; however, the assays were not able to definitively detect any of the targets in the current configuration of the SpinDx. The analytical matrix was rather gel-like, and adding 3 uL of the sample-matrix mix to the port on the disk was difficult and not precise. Sandia supplied a specific pipet and plastic tips to load the device, but the sample loading step could still be improved. The operation of the device was guided by software on a laptop computer connected wirelessly to the SpinDx. Because of concerns about the software measurement of analytical beads after the "spin", Sandia provided calibration beads. However, the analytical software indicated either the calibration beads did not run properly in the disks or the detection algorithm was errant. The battery re-charger connection interferes with closing of the device lid. The system's sample matrix loading and the analytical software both seemed in need of improvement for environmental sample analysis.

The SpinDx was assessed by the Mobile laboratory operators, but not by the field operators. Because of some difficulty in mixing the sample with analytical matrix and pipeting into the disk port, as well as running the control software, the operators rated the "Ease of Use" and "Data" viewing and interpreting attributes as being only FAIR. The operators rated "Training Simplicity", "Safety" and "Maintenance" all as EXCELLENT. The operators also were favorable to the overall design of the instrument and the short musical tune that denotes completion of an assay.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program.

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	N/A	○	N/A
Yersinia pestis	N/A	○	N/A
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	N/A	○	N/A
Yersinia pestis	N/A	○	N/A
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

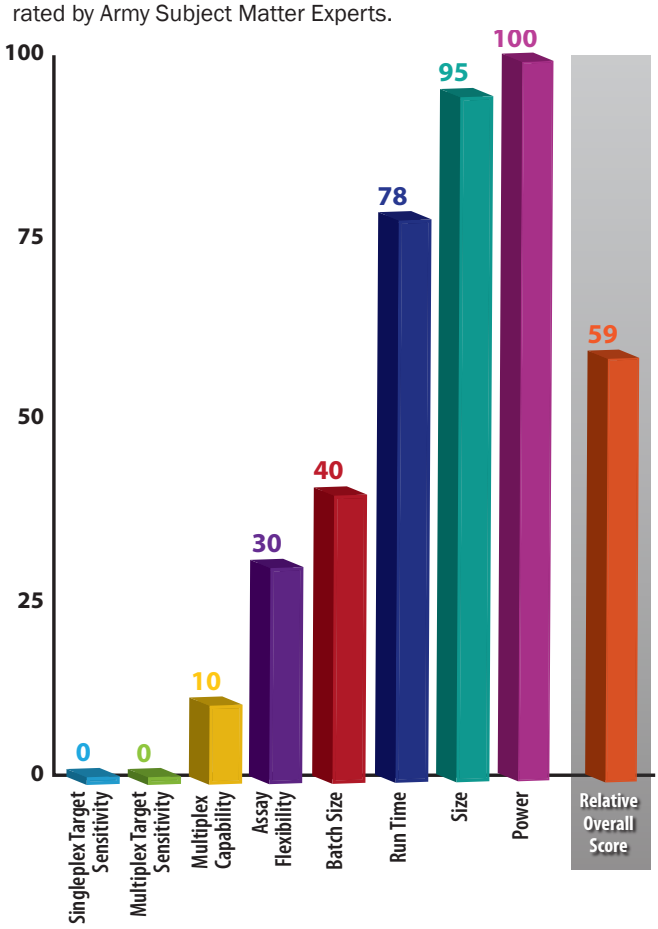
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

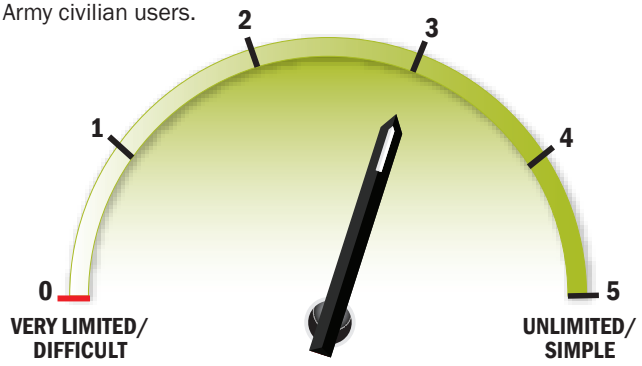
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



SPIRIT™

by Seattle Sensors Corporation

System Specifications

Vendor: Seattle Sensors Corporation

Website: www.seattlesensors.com

System Cost: $35,000.00

Assay Cost: $423.00

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: One

Assay Shelf Life: 6 months at 4 °C

Sample Size Required: 100–150 µL

Type of Detection: Antibody

Time to Result: 5–10 minutes

System Weight: 3 lbs

Operating Range: 59–86 °F (15–30 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). Software is not yet final. System not tested to full capability.

System Description

The SPIRIT by Seattle Sensors Systems Corporation is a shoe-box sized biological agent identifier that uses surface plasmon resonance (SPR) to detect and measure binding, such as between an antibody and a specific bacteria. The SPIRIT has condensed the research laboratory technique of SPR to a portable device capable of detecting bacteria, viruses or toxins from complex samples by utilizing Texas Instruments' Spreeta SPR chips. A laptop computer is used to control the peristaltic pumps and valves and to regulate the flow of sample and buffer onto the SPR flowcells such that each sample may be analyzed within 25 minutes. The operator monitors the SPR signals through a graphic display and post-run data analysis. The system allows for regeneration of the Spreeta chip surfaces, such that up to 100 samples may be analyzed before the chip must be replaced. Among the mobile and man-portable detection systems, SPIRIT has relatively fast assay times, a small footprint and battery-power.

Test Bed Review

Seattle Sensors Systems developed assays for all 5 test agents through binding antibodies from the Critical Reagent Program to individual SPR chips. The software version included with the SPIRIT did not allow full functionality; consequently, only singleplex detection capability was assessed on this version of the SPIRIT. The laboratory assessment utilized individual targets on channel 4 and a calibration control on channel 3, while channels 1 and 2 were not configured for data collection in this software release. The set-up, priming and calibration of the SPIRIT took about 30 minutes, while sample data collection took approximately 25 minutes. The SPIRIT was at the lower end of sensitivity among the biological agent identifiers as bacteria samples were detected at 10^7 CFU/mL and Vaccinia virus inconsistently identified at 10^7 PFU/mL. Meanwhile, VEE was not detected at 10^8 PFU/mL or BoNT A at 1 ug/mL. The SPIRIT required some training and practice for the operators to become comfortable with performing assays.

The SPIRIT received generally favorable usability scores from Mobile laboratory operators, with an EXCELLENT and FAIR rating for "Ease of Use" and GOOD or EXCELLENT scores for Data, Training, Safety and Maintenance categories. The operators had some troubles with the injection of sample to the port and experienced inconsistent internal peristaltic pump pressures. The SPIRIT was sensitive to work surface vibrations perturbing the data collection. Of importance to sample size requirements, one operator was concerned that "1 mL of sample was needed" which may use too much of their collected sample.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	No Claim	○	$>1.00x10^{7}$
Yersinia pestis	No Claim	○	$1.00x10^{7}$
Vaccinia	No Claim	○	$1.00x10^{8}$
Venezuelan Equine Encephalitis	No Claim	○	$>1.00x10^{8}$
Clostridium Botulinum Toxin	No Claim	○	$>1.00x10^{3}$
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus anthracis	$>1.00x10^{7}$	○	N/A
Yersinia pestis	$1.00x10^{7}$	○	N/A
Vaccinia	$1.00x10^{8}$	○	N/A
Venezuelan Equine Encephalitis	$>1.00x10^{8}$	○	N/A
Clostridium Botulinum Toxin	$>1.00x10^{3}$	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

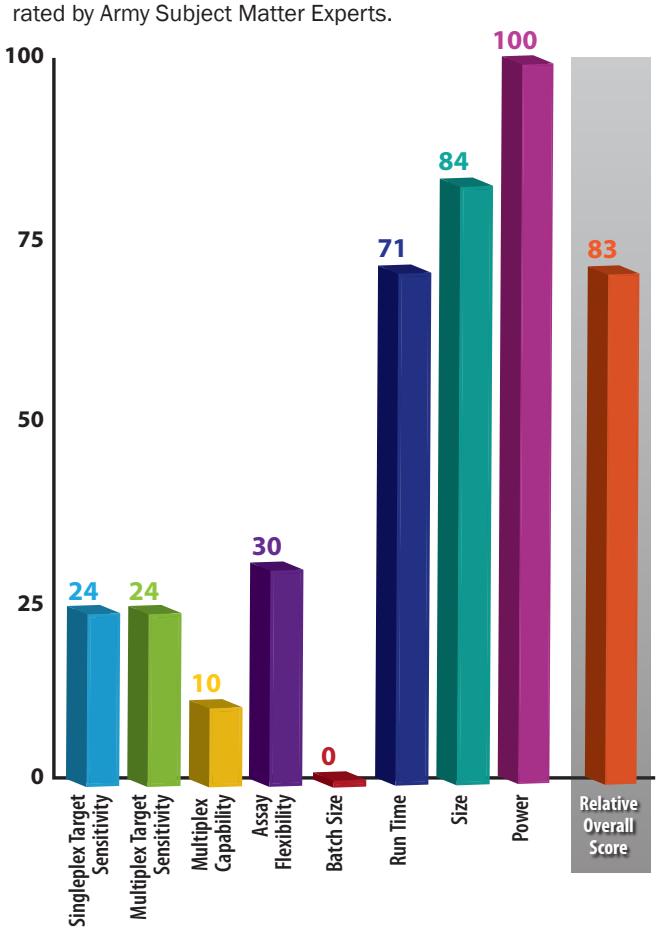
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

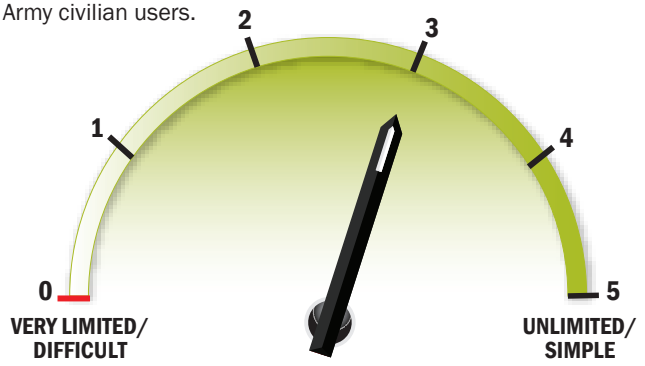
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



TEST BED LABORATORY RESULTS SUMMARIES

FilmArray®

by BioFire™ Diagnostics

System Specifications

Vendor: BioFire Diagnostics, Inc.

Website: www.biofiredx.com

System Cost: $49,500.00

Assay Cost: $185

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: 17 (BioThreat Panel) including multiple targets per agent

Assay Shelf Life: 4-6 months at room temperature

Sample Size Required: 250 µL

Type of Detection: Nucleic acid amplification with end point melt-curve analysis

Time to Result: 65 minutes

System Weight: 20 lbs (systems requires laptop and pouch preparation station not included in weight)

Operating Range: 59 - 86 °F (15 - 30 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab.

System Description

The BioFire FilmArray system is a multiplex PCR system that integrates sample preparation, amplification, detection and analysis. The BioThreat Panel v2.4 pouch stores all the necessary reagents for sample preparation, RT-PCR, PCR and detection in a freeze-dried format. Once an unprocessed sample is injected in to the pouch the FilmArray will extract and purify nucleic acids; perform an initial, large volume multiplex PCR; and complete individual singleplex, second-stage PCR reactions to identify specific targets. Finally the system uses meta analysis of endpoint melt curve data to generate a result for each agent based on the results of one or more targets, each performed in triplicate. Each assay contains internal standards to automatically control for each step of the process. The design of this system requires minimal user training and very little hands on time.

Test Bed Review

The FilmArrays' ability to screen 17 different pathogens (25 targets total) in 60 minutes with sensitivity as low as $5x10^2$ CFU/ml makes this system an asset in any laboratory. Although the FilmArray is not "portable", the unique BioThreat pouch had the ability, during our study, to simultaneously detect 3 targets of *Bacillus anthracis*, 2 targets of *Yersinia pestis*, 2 targets of VEE virus and 2 targets of Orthopox virus. During testing the targets were run as singleplex samples, and then the system was challenged using multiple targets in a single sample. The FilmArray was able to detect all 4 targets with no loss to sensitivity.

During the mobile laboratory, scientists were impressed with the ease of use that the pouch and its associated stand offered. A quick 10 minute training with each end-user allowed them to run the system independently with one scientist commenting, "I wish every system was this easy to run". The biggest impact the system had on the Mobile Lab scientists was its multiplex capability. "For screening an unknown sample, this system would be invaluable", one commented. With time not being an immediate concern for this group, the FilmArray was among their top picks of systems.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX

Agent	Vendor Claimed LOD	Validation of Claimed LOD	Actual LOD
Bacillus anthracis	$5.00x10^2$	⚠	$5.00x10^3$
Yersinia pestis	$5.00x10^1$	✓	$5.00x10^0$
Vaccinia	$1.00x10^2$	⚠	$1.00x10^3$
Venezuelan Equine Encephalitis	$1.00x10^3$	✗	$1.00x10^6$
Clostridium Botulinum Toxin	N/A	○	N/A

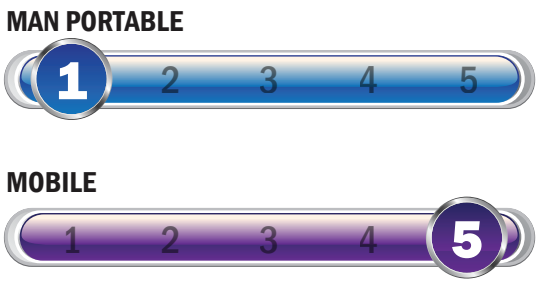
MULTIPLEX

Agent	Actual Singleplex LOD	Actual LOD Achieved	Multiplex LOD
Bacillus antracis	$5.00x10^3$	✓	$5.00x10^3$
Yersinia pestis	$5.00x10^0$	⚠	$5.00x10^1$
Vaccinia	$1.00x10^3$	✓	$1.00x10^3$
Venezuelan Equine Encephalitis	$1.00x10^6$	✓	$1.00x10^6$
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated ⚠ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

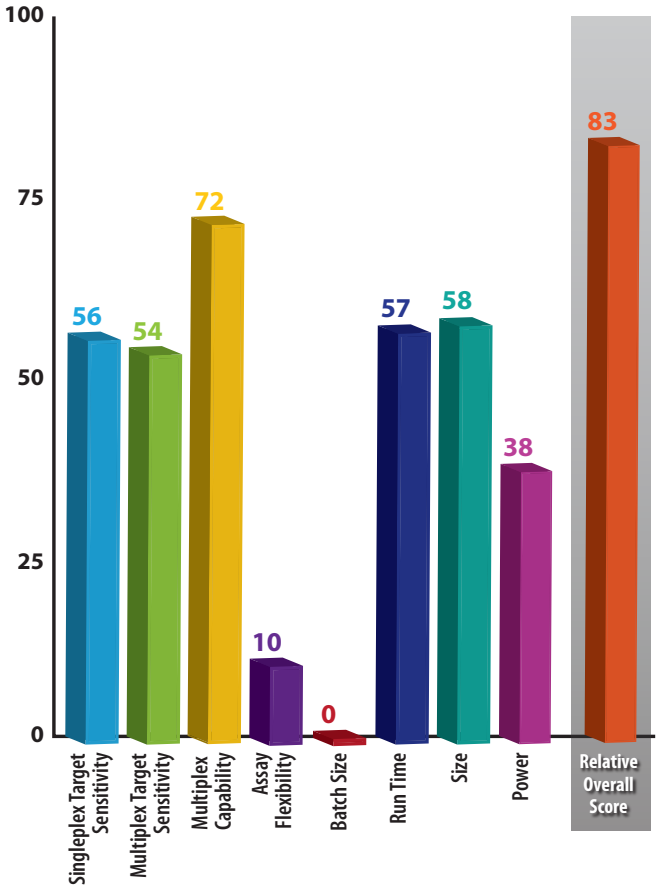
Man Portable and Mobile Usability

Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts



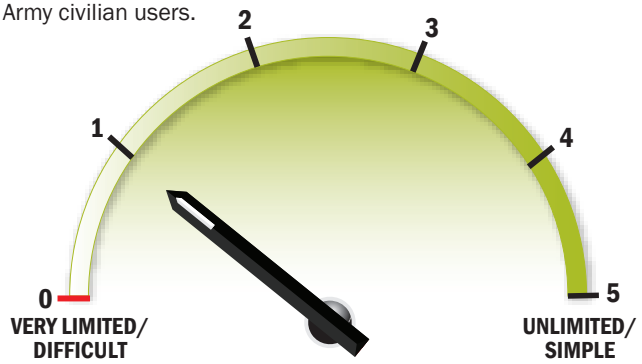
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



BIOFIRE DIAGNOSTICS, INC.

Overview

The BioFire FilmArray system is a multiplex PCR system that integrates sample preparation, amplification, detection, and analysis. The BioThreat Panel v2.4 specific pouch stores all the necessary reagents for sample preparation, real time-PCR (RT-PCR), PCR, and detection in a freeze-dried format. Once an unprocessed sample is injected in to the pouch, the FilmArray will extract and purify nucleic acids; perform an initial, large volume multiplex PCR; and complete individual singleplex, second-stage PCR reactions to identify specific targets. Finally the system uses meta-analysis of endpoint melt curve data to generate a result for each agent based on the results of one or more targets, each performed in triplicate. Each assay contains internal standards to automatically control for each step of the process. The design of this system requires minimal user training and very little hands on time.

The BioThreat Panel v2.4 is a semi-automated assay format for which the user mixes the sample into a dilution matrix. The sample mix and a separate rehydration buffer are drawn into syringes and injected into matching color-coded ports on the assay cassette. Sample preparation, injection of the cassette, and software setup is completed in approximately two minutes. The run-time of the BioThreat Panel v2.4, including automated data analysis, is 65 minutes. BioFire currently has two panels that are FDA-cleared (Respiratory Panel, Blood Culture Identification Panel); however, for this evaluation the newer BioThreat panel, designed to detect 17 pathogens and 25 different targets, was used.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Gamma-irradiated *Bacillus anthracis* spores were diluted to $5x10^1$, $5x10^2$, and $5x10^3$ Colony Forming Units (CFU)/mL in Phosphate Buffered Saline (PBS) solution then tested, in triplicate, using the BioThreat Panel v2.4 assay pouch. Approximately 210 microliters (µL) of sample was transferred to the sample buffer and injected into the pouch which then draws in a pre-determined aliquot of the sample/sample buffer mixture by vacuum. Although the vendor stated the LOD to be $5x10^2$ CFU/mL, consistent detection required a 10-fold increase to $5x10^3$ CFU/mL.

Table 6. *Bacillus anthracis* LOD

Concentration (Colony Forming Units (CFU)/mL)	Total CFU	Total Genome Equivalents (GE)	Results by Loci (Positives/Total Runs)			Result by Agent
			Chromosome	pX01	pX02	*B. anthracis*
$5.00x10^3$	**$1.05x10^3$**	**$2.49x10^3$**	**3/3**	**3/3**	**3/3**	**3/3**
$5.00x10^{2*}$	$1.05x10^2$	$2.49x10^2$	1/3	0/3	2/3	0/3
$5.00x10^1$	$1.05x10^1$	$2.49x10^1$	0/3	0/3	0/3	0/3

Vendor Claimed LOD: $5.00x10^2$ CFU/mL

Yersinia pestis

Gram negative, rod-shaped bacterium

Gamma-irradiated *Yersinia pestis* cells were diluted to $5x10^0$, $5x10^1$, and $5x10^2$ CFU/mL in PBS then tested, in triplicate, using the BioThreat Panel v2.4 assay pouch. Approximately 210µL of sample was transferred to the sample buffer and injected in to the loading dock which then draws in a pre-determined aliquot of the sample mixture by vacuum. The vendor claimed LOD of this assay was $5x10^1$ CFU/mL, however testing 5 CFU/mL was successfully detected.

Table 7. *Yersinia pestis* LOD

Concentration (CFU/mL)	Total CFU	Total GE	Results by Loci (Positives/Total Runs)		Result by Agent
			pPCP1	pMT1	*Y. pestis*
$5.00x10^2$	$1.05x10^2$	$1.45x10^2$	3/3	3/3	3/3
$5.00x10^{1*}$	$1.05x10^1$	$1.45x10^1$	3/3	1/3	3/3
$5.00x10^0$	**$1.05x10^0$**	**$1.45x10^0$**	**3/3**	**0/3**	**3/3**

*Vendor Claimed LOD: $5.00x10^1$ CFU/mL

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Gamma-irradiated VAC virus was diluted to $1x10^1$, $1x10^2$, and $1x10^3$ PFU/mL in PBS then tested, in triplicate, using the BioThreat Panel v2.4 assay pouch. The sample volume transferred to the Sample Buffer was measured to be approximately 210µL. The system draws in a pre-determined aliquot of the sample mixture by vacuum. The vendor stated LOD of this assay is $1x10^2$ PFU/mL, however 10-fold more sample, $1x10^3$ PFU/mL, was required for consistent detection in this study.

Table 8.Vaccinia LOD

Concentration (Plaque Forming Units (PFU)/mL)	Total PFU	Total GE	Results by Loci (Positives/Total Runs)		Result by Agent
			OPX2	VAR3	VAC
$1.00x10^3$	**$2.09x10^2$**	**$3.02x10^2$**	**3/3**	**3/3**	**3/3**
$1.00x10^{2*}$	$2.09x10^1$	$3.02x10^1$	1/3	1/3	1/3
$1.00x10^1$	$2.09x10^0$	$3.02x10^0$	0/3	0/3	0/3

*Vendor Claimed LOD: $1.00x10^2$ PFU/mL

Venezuelan Equine Encephalitis

+ sense single-stranded ribonucleic acid (ssRNA), Alphavirus

Gamma-irradiated VEE virus was diluted to $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, and $1x10^6$ PFU/mL in PBS then tested, in triplicate, using the BioThreat Panel v2.4 assay pouch. The sample volume transferred to the Sample Buffer was measured to be approximately 210µL. The system draws in a pre-determined aliquot of the sample mixture by vacuum. The vendor claimed LOD of this assay was $1x10^3$ PFU/mL, however 1000-fold more sample, $1x10^6$ PFU/mL than the $1x10^3$ PFU/mL LOD described by the vendor.

Table 9. Venezuelan Equine Encephalitis LOD

Concentration (PFU/mL)	Total PFU	Total GE	Results by Loci (Positives/Total Runs)		Result by Agent
			MP2	RC3	VEE
$1.00x10^6$	**$2.09x10^5$**	**$2.09x10^5$**	**3/3**	**3/3**	**3/3**
$1.00x10^5$	$2.09x10^4$	$2.09x10^4$	2/3	1/3	2/3
$1.00x10^4$	$2.09x10^3$	$2.09x10^3$	1/3	0/3	1/3
$1.00x10^{3*}$	$2.09x10^2$	$2.09x10^2$	0/3	0/3	0/3
$1.00x10^2$	$2.09x10^1$	$2.09x10^1$	0/1	0/1	0/1

*Vendor Claimed LOD: $1.00x10^3$ PFU/mL

Multiplex Testing

Based on the results of individual singleplex target detection assessments, samples were prepared containing agents at the following concentrations: *Bacillus anthracis* at $5.00x10^3$ CFU/mL, *Yersinia pestis* at $5.00x10^0$ CFU/mL, VAC at $1.00x10^3$ PFU/mL, and VEE at $1.00x10^6$ PFU/mL. All four targets were detected in all three samples with the exception of *Yersinia pestis,* which was only detected in two of three samples. Fresh samples were prepared with the concentration of *Yersinia pestis* increased 10-fold while all other target concentrations remained the same. In the repeat testing, all three samples were reported positive for all four agents.

Table 10. Multiplex Evaluation

Agent	Concentration (CFU or PFU/mL)	Total CFU or PFU	Total GE	Results by Loci (Positives/Total Runs)			Result by Agent
				Chrom	pXO1	pXO2	*B. anthracis*
B. anthracis	$5.00x10^3$	$1.05x10^3$	$2.49x10^3$	6/6	6/6	6/6	6/6
				pPCP1		pMT1	*Y. pestis*
Y. pestis	$5.00x10^0$	$1.05x10^0$	$1.45x10^0$	2/3		0/3	2/3
	$5.00x10^1$	$1.05x10^1$	$1.45x10^1$	3/3		3/3	3/3
				OPX2		VAR 3	Orthopox
VAC	$1.00x10^3$	$2.09x10^2$	$3.02x10^2$	5/6		3/6	6/6
				MP2		RC3	VEE
VEE	$1.00x10^6$	$2.09x10^5$	$2.09x10^5$	6/6		6/6	6/6

Discussion

Call Assignments

One concern observed with the system was the arbitrary manner in which positive and negative system calls of pathogen identifications are made. In most cases, a positive call was made if any single locus was detected. Anecdotal experience with large surveillance programs such as the Department of Homeland Security's (DHS) BioWatch and JPEO-CBD efforts indicates that single locus detection of bacterial

pathogens has an unacceptable high false positive rate, especially in environmental samples. This is particularly true when the definitive locus is present on plasmids, which are often shared between bacteria via horizontal gene transfer. As a result, ambiguous results are sometimes generated but not reported as "ambiguous". For example, a low-level detection event identified by the single most sensitive locus PCR reaction could be confused with a singleplex positive result from a genetic near-neighbor sharing a genetic target with the organism of interest.

This approach also discards one of the most desirable features of multiplex detection: the power of joint probability distributions of multiple positive, independently developed assays to provide higher confidence results than possible with any single assay alone. For example, three independent assays for a given pathogen might have a false positive rate of 10^{-2}, but the simultaneous detection of all three loci would result in a calculated false positive rate for the organism of 10^{-6}.

It is worth noting that broad and inclusive detection of highly divergent organisms, such as ssRNA viruses, may require multiple loci to capture the entire detection space. For example, one locus may detect one serotype, while another may be required to detect other serotypes of the same species. In this case, each locus would be considered as a subset of a single assay for simple terms of inclusivity and any positive result of this assay would be considered a positive result.

Plasmid and Chromosomal Targets

The utility of using both plasmid and chromosomal PCR targets has the advantage, as noted above, of high confidence identification of the organism and the ability to look for multiple virulence determinants found on separate elements of the genomic architecture. Because *Bacillus* plasmids may transfer between species without conference of virulence, reliance on plasmids alone as PCR targets may result in higher false positive rates. In the BioThreat Panel v2.4, the *B. anthracis* assay includes what has become a standard configuration: a chromosomal target and one target for each of the two virulence plasmids (pXO1 and pXO2), allowing for simultaneous high confidence detection of the organism and the known pathogenicity islands. However, the *Y. pestis* assay has only two of the three virulence plasmids, and omits any chromosomal targets. The addition of targets on the third virulence plasmid (pCD1) and the chromosome would be of significant advantage for a more complete and actionable assay.

One distinct benefit of targeting plasmids is the fact that they are usually multi- or high-copy replicons, which increases the sensitivity of these assays for a given CFU of input material. Unfortunately, the sensitivity of the various BioThreat Panel v2.4 PCR assays does not seem to reflect the expected increase in sensitivity for plasmid targets as expected. In the *Bacillus anthracis* assay, there is a suggestion that the pXO2 assay is indeed slightly more sensitive than the chromosomal target, but additional testing with finer graduations of template concentrations and more replicates would be required to confirm this preliminary finding. However, even with this limited data, it is clear that the pXO1 assay is approximately 10-fold less sensitive that the other two assays. The disparity in expected PCR performance (more than an order of magnitude without considering the differences in template concentrations between the chromosome and plasmid) raises some concern that the optimization of the individual PCR reactions requires some additional work.

Assay Sensitivity

The laboratory determined assay LOD for VEE is approximately three orders of magnitude higher than that stated in the product literature. The two bacterial assays demonstrated sensitivity concordant with

vendor claims, and the dsDNA virus target (VAC) did not have a published LOD. It is possible that the lack of sensitivity of the VEE assay is related to either variability of the input template (viral RNA preparations can vary significantly as a function of total target RNA to PFU ratios in different production lots) or the efficiency of the RNA purification and reverse transcription to complementary DNA (cDNA). Additional investigation to determine the sensitivity of cDNA versus native RNA targets and more information from the assay developers may shed light on the disparity observed in assay performance.

The observed sensitivity of the VAC assay was moderately less in multiplex versus the singleplex format. All other assays seemed to be equivalently sensitive in either format. One of the assays (VAR3) was particularly affected (only 50% detection rate at the LOD in multiplex versus 100% in singleplex). This phenomenon is not unexpected because of known issues with competition for reagents that require precise balancing of simultaneous reactions in multiplex PCR. However, some additional multiplex PCR optimization, and the potential need to screen additional pan-orthopox assays, will be required to demonstrate the same level of even performance shown with the other organisms.

RAZOR®

by BioFire Diagnostics, Inc.

System Specifications

Vendor: BioFire Diagnostics, Inc.

Website: www.biofiredx.com

System Cost: $38,500.00

Assay Cost: $200.00

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: 10

Assay Shelf Life: 1 year at room temperature

Sample Size Required: 250 µL

Type of Detection: Nucleic Acid

Time to Result: 25 minutes

System Weight: 11 lbs

Operating Range: 32–104 °F (0–40 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab and field.

System Description

BioFire's RAZOR EX is a field PCR unit that uses pouches pre-loaded with freeze-dried PCR reagents for the detection and identification of biological pathogens and biothreat agents. Each kit contains all of the items necessary for sampling, sample preparation and real-time PCR. Each kit includes items needed for collecting and loading the sample. Once samples are loaded into the pouch with cannula-tipped syringes they are dispensed automatically in to the wells, requiring no precise measuring. BioFire has assays available for CDC defined Category A and B Biothreat pathogens. One pouch will test for 3 to 10 different agents, depending on configuration, and includes internal controls to validate the integrity of the test. BioFire's pouches and its associated kit components are manufactured under a cGMP quality system. This testing used The TEN™ 10 Target Screen Kit to detect 2 out of 4 desired targets, *Bacillus anthracis* and *Yersinia pestis*, and verify vendor claims of limits of detection (LOD). Additionally, the Botulinum toxin preparation was tested for residual C. botulinum DNA.

Test Bed Review

The RAZOR was one of the few systems in this test that had been designed as a field-ready system. Because of this, the expectation that it would outperform the others was there, however the ease of use did not meet up with the end users requirements.

In the laboratory the system performed comparable to the other PCR systems, with LOD's down to 102, for its *Yersinia pestis* assay. Unfortunately, BioFire's RAZOR is considered a "closed" system, the company was not willing to develop new assays for this study therefore only 2 (*Yersinia pestis* and *Bacillus anthracis*) of the desired 5 targets were tested. This closed system is an unfavorable characteristic for military applications that are challenged with new threats and require new assays at any given time.

During the field testing, soldiers had difficulty manipulating the pouch and found the barcode scanner difficult to use. One commented, "This system is tedious". Another concern for the soldiers in the field was the difficulty they had reading the screen in the daylight. One soldier noted "the brightness option does not help in the bright sunlight". Overall the field ready system isn't as ready as it appears. In full MOPP gear the consumables gave the greatest challenge even for the simplest of tasks, such as opening a box.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	$1.00x10^3$	⚠	$1.30x10^4$
Yersinia pestis	$1.00x10^2$	⚠	$1.30x10^3$
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$1.30x10^4$	✓	$1.30x10^4$
Yersinia pestis	$1.30x10^3$	✓	$1.30x10^3$
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated ⚠ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

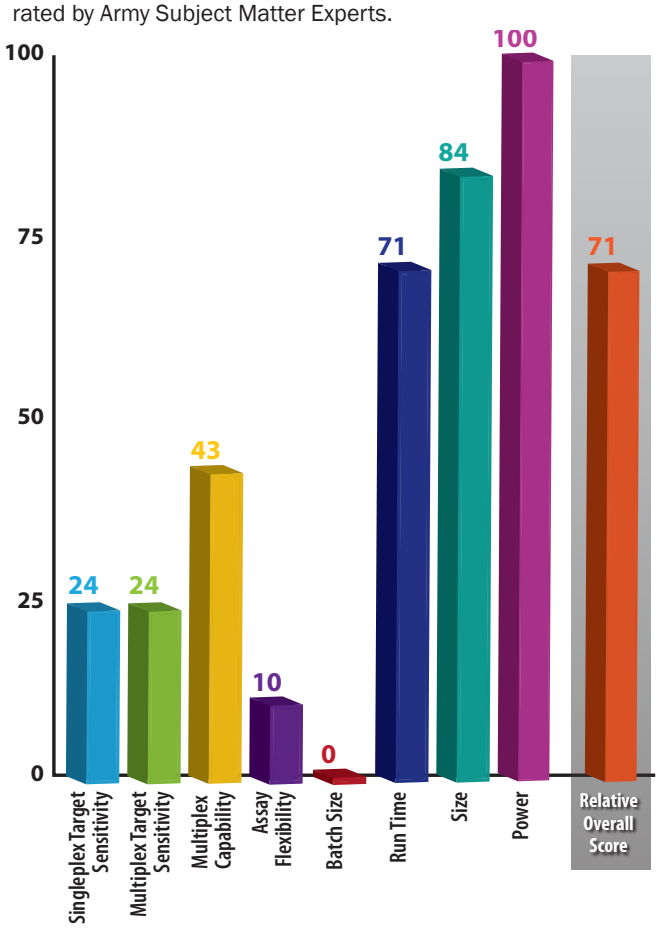
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

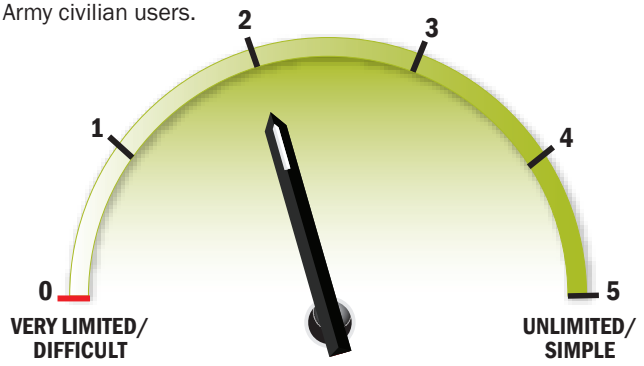
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Overview

BioFire's RAZOR EX is a field PCR unit that uses pouches pre-loaded with freeze-dried PCR reagents for the detection and identification of biological pathogens and biothreat agents. Each kit contains all of the items necessary for sampling, sample preparation, and real-time PCR. Each kit includes items needed for collecting and loading the sample. Once samples are loaded into the pouch with cannula-tipped syringes they are dispensed automatically into the wells, requiring no precise measuring. BioFire has assays available for CDC-defined Category A and B Biothreat pathogens. One pouch will test for three to 10 different agents, depending on configuration, and includes internal controls to validate the integrity of the test. BioFire's pouches and its associated kit components are manufactured under a Current Good Manufacturing Practices (cGMP) quality system. This testing used The TEN® Target Screen Kit to detect two out of four desired targets, *Bacillus anthracis* and *Yersinia pestis*, and verify vendor claims of LOD. Additionally, the Botulinum toxin preparation was tested for residual *C. botulinum* DNA.

The RAZOR EX contains a barcode reader on the rear of the device. A user selects the option to run an assay from the device home screen then is prompted to scan the barcode of the assay. Once the assay is recognized by the instrument the user is prompted to prepare the sample and load it into the kit through onscreen step by step instructions. First, the user draws an aliquot of a negative control (provided in each kit) into a syringe and pushes the plastic cannula into the marked port of the pouch. Vacuum draws the required amount of liquid into the wells to rehydrate negative and inhibition controls. Second, the user mixes the sample into 5mLs of sample diluents (also provided in each kit and labeled as "unknown"), draws a portion of the sample mixture into a syringe, and inserts the plastic cannula into the marked port. Again, vacuum draws the requisite amount into the pouch. Lastly, the user removes a comb from around the plungers, rotates the plungers, and pushes them down to force the rehydrated reagents into the assay strips which contain the primer and probe oligonucleotides. Preparing the sample, injecting the pouch, and twisting the plungers requires approximately five minutes. Once the properly prepared pouch is installed onto the device, results for all ten targets are available within 25 minutes.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Gamma-irradiated *Bacillus anthracis* spores were serially diluted in 10-fold increments into water then tested, in triplicate, using The TEN® Target Screen Kit. The sample volume was transferred to the "unknown" bottle using the transfer pipet included in the kit. The instructions and kit information list the volume transferred to be 0.5mL although it was determined to be approximately 265µL based on the weight of water transferred. Therefore, the sample was further diluted nearly 20-fold before being loaded into the pouch. The requisite amounts of water for negative and inhibition controls as well as of the sample itself were drawn into the pouch by vacuum. Although the vendor stated LOD of the assay for *B. anthracis* is $1x10^3$ CFU/mL, 10-fold more was required for consistent detection of *B. anthracis* in this study.

Table 11. *Bacillus anthracis* LOD

Concentration (Colony Forming Units (CFU)/mL)	Total CFU	Total GE	Results by Loci (Positives/Total Runs)
			pX02
$1.30x10^5$	$3.45x10^4$	$8.20x10^4$	3/3
$1.30x10^4$	**$3.45x10^3$**	**$8.20x10^3$**	**3/3**
$1.30x10^{3*}$	$3.45x10^2$	$8.20x10^2$	2/3

*Vendor Claimed LOD: $1.00x10^3$ CFU/mL

Yersinia pestis

Gram negative, rod-shaped bacterium

Gamma-irradiated *Yersinia pestis* cells were serially diluted in 10-fold increments into water then tested, in triplicate, using The TEN® Target Screen Kit. The sample volume was transferred to the "unknown" bottle using the transfer pipet included in the kit. The instructions and kit information list the volume transferred to be 0.5mL although it was determined to be approximately 265µL based on the weight of water transferred. Therefore, the sample was further diluted nearly 20-fold before being loaded into the pouch. The requisite amounts of water for negative and inhibition controls as well as of the sample itself were drawn into the pouch by vacuum. Although the vendor stated LOD of the assay for *Y. pestis* is $1x10^2$ CFU/mL, 10-fold more was required for consistent detection in this study.

Table 12. *Yersinia pestis* LOD

Concentration (CFU/mL)	Total CFU	Total GE	Results by Loci (Positives/Total Runs)
			pPCP1
$1.30x10^4$	$3.45x10^3$	$4.77x10^3$	3/3
$1.30x10^3$	**$3.45x10^2$**	**$4.77x10^2$**	**3/3**
$1.30x10^{2*}$	$3.45x10^1$	$4.77x10^1$	2/3

*Vendor Claimed LOD: $1.00x10^2$ CFU/mL

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Gamma-irradiated VAC virions were serially diluted in 10-fold increments into water then tested, in triplicate, using The TEN® Target Screen Kit. The sample volume was transferred to the "unknown" bottle using the transfer pipet included in the kit. The instructions and kit information list the volume transferred to be 0.5mL although it was determined to be approximately 265µL based on the weight of water transferred. Therefore, the sample was further diluted nearly 20-fold before being loaded into the pouch. The requisite amounts of water for negative and inhibition controls as well as of the sample itself were drawn into the pouch by vacuum. Upon obtaining all negative detections for the $1.30x10^5$ PFU/mL sample, 100 times more sample was tested. This sample was also negative. The vendor noted that the assay had been modified from a pan- orthopox assay to a Variola-specific assay.

Table 13. Vaccinia LOD

Concentration (Plaque Forming Units (PFU)/mL)	Total PFU	Total GE	Results by Loci (Positives/Total Runs)
			VAC
$1.30x10^7$	$3.45x10^6$	$4.97x10^6$	0/1
$1.30x10^5$	$3.45x10^4$	$4.97x10^4$	0/3

*Vendor Claimed LOD: N/A

Venezuelan Equine Encephalitis

+ sense ssRNA, Alphavirus

No Assay Available

Clostridium botulinum Type A Toxin

Protein toxin

The TEN® Target Screen Kit includes an assay for *Clostridium botulinum*, although the target is DNA rather than the active toxin used in this assessment. The BoNT A, as supplied by Metabiologics, is produced in its native organism; therefore, the agent was tested to determine whether the system could detect residual *C. botulinum* DNA in the toxin preparation. The device did not detect residual DNA, which is consistent with information obtained from Metabiologics that the products have very low A_{260} measurements.

Table 14. BoNT A LOD

Concentration (Nanograms (ng)/mL)	Total Nanograms	Results by Loci (Positives/Total Runs)
		Unknown
$1.00x10^3$	250	0/1
$1.00x10^2$	25	0/3

*Vendor Claimed LOD: N/A

Multiplex Detection

Based on the results of individual singleplex target detection assessments, separate samples were prepared containing *B. anthracis* at $2.60x10^4$ CFU/mL and *Y. pestis* at $2.00x10^3$ CFU/mL. The two samples were combined in equal portions to create the test sample, which was tested in triplicate. Both *B. anthracis* and *Y. pestis* were detected in all three samples.

Table 15. Multiplex Evaluation

Agent	Concentration (CFU/mL)	Total CFU	Total GE	Results by Loci (Positives/Total Runs)
B. anthracis	**$1.30x10^4$**	**$3.44x10^3$**	**$8.19x10^3$**	**3/3**
Y. pestis	**$1.00x10^3$**	**$2.65x10^2$**	**$3.68x10^2$**	**3/3**

Discussion

Call Assignments

Instrument calls are assigned by single locus amplification and real-time, exponential single color fluorescence measurements. A single sample can be screened against up to ten loci/agents in approximately 30 minutes including integrated sample preparation. Aliquots of the purified templates are diverted into individual channels for separate single-color real-time PCR reactions, eliminating issues of PCR reaction interference and/or color separation issues that might provide lower sensitivity and specificity. However, anecdotal experience with large surveillance programs such as DHS' BioWatch and JPEO-CBD would indicate that single locus detection of bacterial pathogens has an unacceptable high false positive rate, especially in environmental samples. As configured, the system is appropriate for low-throughput applications such as screening suspicious bulk materials/high titer clinical samples for presumptive presence of a threat agent. In circumstances with a known and verified threat (such as an existing outbreak), this would be sufficient for a positive assignment. For routine surveillance, multi-locus and/or orthogonal confirmation with culture or immunoassay would be required for verification.

Plasmid and Chromosomal Targets

Both the *B. anthracis* (pXO2) and *Y. pestis* (pPCP1) assays identify plasmid targets. For singleplex assays, this has both advantages and disadvantages. Sensitivity of plasmid targets (especially smaller and potentially higher-copy replicons such as pPCP1) is usually superior to that achievable with chromosomal targets. However, the known issues of horizontal gene transfer and plasmid sharing often seen between closely related near neighbor species can cause problems with specificity and generate false-positive reactions and instrument calls. Due to the limited testing (only two bacteria and a single viral locus with no appropriate template control available) and lack of additional assay targeted at other discrete loci, there is no way to evaluate specificity versus sensitivity trade-offs in this study.

The choice to include only a Variola-specific assay is problematic for two reasons. The vendor did not provide the positive control (plasmid containing expected amplicon) and federal regulations do not permit the distribution of the Variola genome, which did not allow for laboratory testing to verify vendor claims. This unfortunately also does not allow for simultaneous detection of other known dangerous pathogens in the orthopox family (Monkeypox, Camelpox, and Vaccinia). Inclusion of a pan-orthopox assay would have given a broader representation of poxvirus pathogen detection, as well as allowing for routine testing with unregulated materials.

Assay Sensitivity

For a PCR platform, the sensitivity of the RAZOR EX is less than the theoretical limit of single copy detection. A major source of sensitivity loss is the initial dilution of sample. Another specific source of sensitivity loss is the sample splitting into 10 individual assay amplifications. Subsampling in this manner automatically reduces sensitivity by 10-fold, and provides for a sharp increase in false negatives because of unfavorable Poisson distributions at low concentrations of target DNA. This can potentially be overcome by targeting high-copy number loci, and would not be an issue when sampling bulk or high-

titer samples. One benefit of this approach is the reduction in assay interference normally present in a multiplex PCR format.

The LOD of the most sensitive assay tested (*Y. pestis*) is between 50-500 genome equivalents. Ignoring plasmid copy number and allowing for moderate sample loss due to bacterial lysis inefficiency, the actual sensitivity in the individual PCR reactions is more likely on the order of 10 genome equivalents. Therefore, the sensitivity of RAZOR EX is limited by sample preparation and amplification inefficiencies. Considering the three sources of sensitivity loss listed above, the detection capabilities of the RAZOR EX are likely very close to single copy limits.

Genedrive™

by Epistem, Inc.

System Specifications

Vendor: Epistem, Inc.

Website: www.epistem.co.uk

System Cost: $4,000

Assay Cost: Price Request (Price estimated to be $85/assay, subject to quantity)

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: 4 per Assay

Assay Shelf Life: Unknown

Sample Size Required: 20 µL

Type of Detection: Nucleic Acid

Time to Result: 60 minutes

System Weight: 1.2 lbs

Operating Range: 32–131 °F (0–55 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System is still in prototype stage. Some components need refinement.

System Description

The Genedrive is a fully integrated, endpoint PCR-based platform. Genedrive's proprietary "hybrid thermal engine" allows faster cooling rates and shorter annealing times relative to conventional PCR devices. The system is capable of performing ultra fast PCR cycling of 30 cycles in as little as 17 minutes and is controlled by a single button. The Genedrive was designed to be a highly cost-effective way of moving molecular diagnostics from the laboratory to the point of need across several markets including government. Epistem currently has a *Mycobacterium tuberculosis* IVD assay for the Genedrive that has received European approval. For this testing Epistem developed multiplex assays to detect *Bacillus anthracis*, *Yersinia pestis*, Vaccinia and Venezuelan Equine Encephalitis and a singleplex assay to detect active Botulinum neurotoxin type A.

Test Bed Review

On paper, the Genedrive's low cost, small footprint and short run time were appealing; unfortunately, the assays did not meet expectations and required modified testing procedures to achieve successful data. After several iterations, including eliminating the sample preparation paper and making internal hardware structure changes, the system was able to detect approximately 1.66×10^5 CFU/mL in the *Yersinia pestis* assay. However, *Bacillus anthracis* and *Vaccinia* detection limits were 100 fold higher and the device couldn't run the VEE assay because of firmware shortcomings. The 60 minute run time was unexpected, since the system was projected to perform 30 cycles in 17 minutes. Genedrive also produced false positives and inconsistent results. Our testing demonstrated that unrefined sample preparation and assay reagents also contributed to poor performance.

Field-users were impressed with the size of the system, but little else. Surprisingly, the sample preparation created the hardest task of manipulating several tubes. One user commented, "Anything that requires several steps would require two operators for set up and therefore would not be used in the field". One soldier noted that the humid weather prevented the sample preparation cards from completely drying. Another user commented "The device needs some method of data accessibility and storage such as a thumb drive for chain of evidence."

Genedrive did not perform well for bacterial and virus detection but was the only PCR-based system that could detect the presence of toxin. The system design is promising and grabs one's attention, but assay design and sample preparation require additional development, putting this system near the bottom for performance in our testing.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = GE, Virus = GE, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	10	✗	69,000
Yersinia pestis	10	✗	200
Vaccinia	10	✗	34,000
Venezuelan Equine Encephalitis	10	○	Not Tested
Clostridium Botulinum Toxin	No Claim	○	10,000
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	69,000	○	N/A
Yersinia pestis	200	○	N/A
Vaccinia	34,000	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	10,000	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

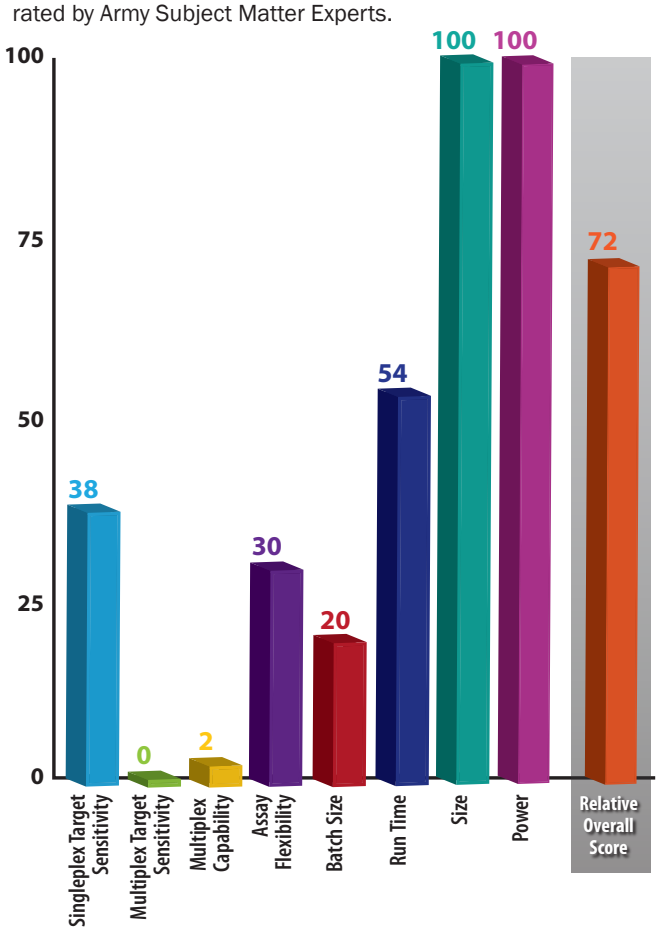
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

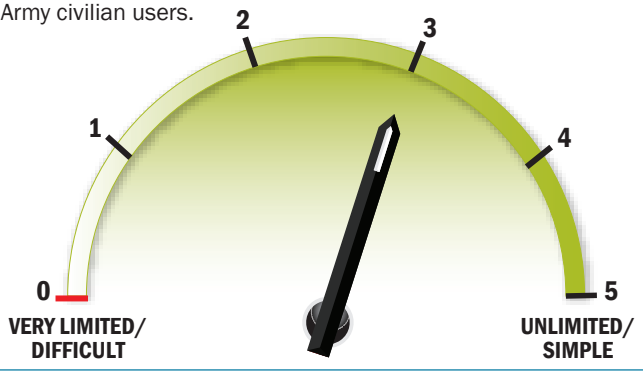
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



epistem

Overview

The Genedrive™ is a fully integrated, endpoint PCR-based platform. Genedrive's proprietary "hybrid thermal engine" allows faster cooling rates and shorter annealing times relative to conventional PCR devices. The system is capable of performing ultra-fast PCR cycling of 30 cycles in as little as 17 minutes and is controlled by a single button. The Genedrive was designed to be a highly cost-effective way of moving molecular diagnostics from the laboratory to the point of need across several markets including government. Epistem has a *Mycobacterium tuberculosis in vitro* diagnostics (IVD) assay for the Genedrive that received the CE-IVD Mark in Europe. For this testing, Epistem developed multiplex assays to detect *Bacillus anthracis, Yersinia pestis,* Vaccinia and Venezuelan Equine Encephalitis and a singleplex assay to detect active Botulinum toxin type A.

For detection of agent with Epistem's BIOT ID™ Cartridges, 25µL of sample is spotted onto a 1cm disc of 2B BlackBio BlackLight paper and allowed to dry for 10 minutes. Three 1mm discs are removed from the spot with a biopsy punch and one disc is added to each well of the cassette. Additionally, three GE Healthcare Ready-To-Go PuReTaq PCR beads are rehydrated with 20µL water and each transferred to a well of the cassette. The cassettes contain lyophilized primer/probe oligonucleotides in two of the three wells, while the third well is a negative control. Each of the wells containing oligonucleotides contained sets allowing detection of two agents. Preparation of the sample using the BlackLight paper, rehydration of PCR beads, and assembly of the cassettes takes approximately 15 minutes. Once the cassette is loaded into the device, results are ready in 65 minutes. The device displays "undetected OK" or "detected positive." The user must then navigate to a secondary screen and determine whether the melt peaks identified by the software match the expected T_m (melting temperature) although the system, as configured, does not prompt the user to view the data.

Early in the evaluation, potential problems with external light interference, sample preparation and assay stability were identified. To overcome the light issue, the vendor replaced the Genedrive with another model that contained an additional gasket to seal the internal components. The vendor also identified the lyophilization of the BIOT ID reagents as being potentially defective. To remedy the assay stability problems, the vendor provided individual primer and probe mixtures for each assay to replace the BIOT ID cartridges. Further testing demonstrated the BlackLight paper was an inefficient sample preparation medium and caused significant dilution of the target.

The testing procedures were modified as a result of these initial tests. Sample preparation using the BlackLight paper was replaced by testing of purified DNA extracted from inactivated agents and assays were run in cassettes not containing lyophilized primer/probes. Agent specific primers and probes were added from a rehydrated stock. DNA had been purified from each agent in order to test the Tetracore T-COR 4 system, so these DNA samples were used to test the sensitivity of the Genedrive assays. For each test, three Ready-To-Go PuReTaq PCR beads were rehydrated with 20µL water each and transferred to a well of the cassette. One microliter of DNA in TE buffer and one microliter of primer/probe mix in water were added to each cassette well. Assays were considered positive only if the melt peak matched the expected T_m provided by Epistem.

The BoNT A assay, as stated above, is a fluorescence-based assay based on the enzymatic activity of BoNT A. The amount of fluorescence is directly proportional to the amount of active BoNT A in the sample. The assay developed by SRC (Syracuse Research Corporation) is a simple, straightforward assay in which the user mixes the sample into three assay tubes containing a synthetic, fluorescently labeled substrate. The results are available after a one hour isothermal incubation at 37°C as an increase in fluorescence relative to the control tube. Unfortunately, neither SRC nor Epistem provided guidelines for threshold Δ-fluorescence measurements.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Purified DNA extracted from gamma-irradiated *Bacillus anthracis* spores was serially diluted in 10-fold increments into 50 millimolar (mM) Tris HCl, pH 8.0 and evaluated on the Genedrive platform. PCR beads were rehydrated with 20µL water then 1µL primer/probe mix and 1µL sample was added after transferring the reaction mix to cassette. The LOD of the device was determined to be nearly 70,000 GE, 7,000-fold greater than the LOD claimed by Epistem. Considering the size of the sample and reported GE to CFU ratio, this LOD is equivalent to approximately $2.90x10^{7}$ CFU/mL.

Table 16. *Bacillus anthracis* LOD

Concentration (picogram (pg)/uL)	Total pg	Total Genome Equivalents (GE)	Equivalent CFU/mL	Results by Loci (Positives/Total Runs)
				rpoB
$3.90x10^{-1}$	$3.90x10^{-1}$	$6.90x10^{1}$	$2.90x10^{4}$	0/1
$3.90x10^{0}$	$3.90x10^{0}$	$6.90x10^{2}$	$2.90x10^{5}$	0/1
$3.90x10^{1}$	$3.90x10^{1}$	$6.90x10^{3}$	$2.90x10^{6}$	1/3
$3.90x10^{2}$	**$3.90x10^{2}$**	**$6.90x10^{4}$**	**$2.90x10^{7}$**	**3/3**

Vendor Claimed LOD: 10GE

Yersinia pestis

Gram negative, rod-shaped bacterium

Purified DNA extracted from gamma-irradiated *Yersinia pestis* cells was serially diluted in 10-fold increments into 50mM Tris HCl, pH 8.0 and evaluated on the Genedrive. The LOD of the device was determined to be over 200 copies, 20-fold greater than the LOD claimed by Epistem. Considering the size of the sample and reported GE to CFU ratio, this LOD is equivalent to approximately $1.66x10^{5}$ CFU/mL.

Table 17. *Yersinia pestis* LOD

Concentration (pg/uL)	Total pg	Total GE	Equivalent CFU/mL	Results by Loci (Positives/Total Runs)
				pPCP
$1.20x10^{-1}$	$1.20x10^{-1}$	$2.30x10^{1}$	$1.66x10^{4}$	1/3
$1.20x10^{0}$	**$1.20x10^{0}$**	**$2.30x10^{2}$**	**$1.66x10^{5}$**	**3/3**
$1.20x10^{1}$	$1.20x10^{1}$	$2.30x10^{3}$	$1.66x10^{6}$	3/3
$1.20x10^{2}$	$1.20x10^{2}$	$2.30x10^{4}$	$1.66x10^{7}$	1/1

Vendor Claimed LOD: 10 GE

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Purified DNA extracted from gamma-irradiated VAC viral particles was serially diluted in 10-fold increments into 50mM Tris HCl, pH 8.0 and evaluated on the Genedrive. Two rounds of testing were completed because Epistem miscommunicated the expected T_m . Since the device has three wells, the lowest two concentrations were evaluated simultaneously. The gain for well three appears to the set much higher than wells one and two; therefore, the $6.90x10^{-2}$pg/µL sample (run in well three) was positive while the $6.90x10^{-1}$ pg/µL sample (run in well two) was negative. The LOD of the device was determined to be $6.90x10^{0}$ pg/µL because this concentration could be detected in both wells two and three. Well one was reserved for an NTC in all experiments. This LOD is equivalent to approximately 34,000 copies, over 3,000-fold greater than the LOD claimed by Epistem. Considering the size of the sample and reported GE to PFU ratio, this LOD is equivalent to approximately $2.35x10^{7}$ PFU/mL.

Table 18. Vaccinia LOD

Concentration (pg/uL)	Total pg	Total GE	Equivalent PFU/mL	Results by Loci (Positives/Total Runs)
				pPCP
$6.90x10^{-2}$	$6.90x10^{-2}$	$3.38x10^{2}$	$2.35x10^{5}$	1/1
$6.90x10^{-1}$	$6.90x10^{-1}$	$3.38x10^{3}$	$2.35x10^{6}$	2/3
$6.90x10^{0}$	**$6.90x10^{0}$**	**$3.38x10^{4}$**	**$2.35x10^{7}$**	**3/3**
$6.90x10^{1}$	$6.90x10^{1}$	$3.38x10^{5}$	$2.35x10^{8}$	2/2
$6.90x10^{2}$	$6.90x10^{2}$	$3.38x10^{6}$	$2.35x10^{9}$	3/3
$6.90x10^{3}$	$6.90x10^{3}$	$3.38x10^{7}$	$2.35x10^{10}$	2/2
$7.50x10^{4}$	$7.50x10^{4}$	$3.38x10^{8}$	$2.35x10^{11}$	3/3

Vendor Claimed LOD: 10 GE

Venezuelan Equine Encephalitis

+ sense ssRNA, Alphavirus

The VEE assay was not run due to lack of a pre-programmed RT-PCR step in the thermal cycling algorithm.

Clostridium botulinum Type A toxin

Protein toxin

BoNT A complex was serially diluted into PBS then tested with the SRC-developed toxin activity assay. Twenty microliters of each sample was added to each assay tube (denoted C, T1, and T2), each containing 50µL of reagent. The reagent in the C tube was a non-fluorescent control while tubes T1 and T2 contained a synthetic substrate that fluoresces when proteolytically cleaved by BoNT A. Twenty microliters of each sample mixture was transferred to a Genedrive cassette and evaluated for increase in fluorescence during a 1 hour isothermal incubation at 37°C. The LOD of the assay for BoNT A was determined to be 10µg/mL by evaluating the slope of the fluorescence plots.

Table 19. *Clostridium botulinum* toxin Limit of Detection LOD

Concentration (nanogram (ng)/mL)	Total ng	Call	Slope Values (ΔF/Δt)		
			Control	T1	T2
$1.00x10^{2}$	$1.00x10^{-1}$	0/1	-1.06	-0.14	-0.05
$1.00x10^{3}$	$1.00x10^{0}$	0/1	-1.00	-0.04	0.02
$1.00x10^{4}$	**$1.00x10^{1}$**	**3/3**	**-.092^{T}**	**1.21^{T}**	**0.93^{T}**

T Average of Triplicates

Vendor Claimed LOD: N/A

Multiplex Detection

Manufacturing issues with the BIOT ID cartridges were identified early in the device assessment; therefore, all testing was completed with rehydrated primer/probe mixtures in a singleplex format. As tested, no multiplex data was generated.

Discussion

Call Assignments

Individual call assignments are made based upon whether products can be detected by automated, high-resolution melt curve analysis (peak negative derivative of the fluorescence versus temperature curve). If products are detected by the algorithm, the device displays “detected positive” on the screen. However, the user is required to navigate to a secondary screen which displays the melting temperatures of the products and compare the temperatures to parameters supplied by the vendor. This creates issues wherein spurious, non-specific amplifications are detected by the algorithm. This triggers the device to display a positive detection on the results screen but the T_m of the product does not match the expected temperature range–thus resulting in a false positive result. For the purposes of this evaluation, only positive instrument results with a correct amplicon T_m were considered positive for the test agent.

Plasmid and Chromosomal PCR Targets

For the two bacterial organisms, the Epistem Genedrive assays use both chromosomal (*B. anthracis*) and plasmid (*Y. pestis*) targets. Each approach has benefits and weaknesses; plasmids tend to be present at higher copy number and therefore have better sensitivity, while well-selected chromosomal markers tend to be less prone to cross reactivity due to horizontal gene transfer. Using both has distinct advantages, giving high confidence detection and identifying genetic elements required for virulence in a single instrument run. For the viral targets (VEE E2 and VAC F5L), these are both single-copy targets by definition, and specificity would improve with multiple independently validated assays.

The choice of the rpoB gene for *B. anthracis* detection can be problematic in terms of specificity, as it is essentially a housekeeping gene found in most, if not all, bacteria. In fact, like 16S sequences, small variations in rpoB sequence are often used for taxonomic discrimination of bacteria. However, this was a conscious decision by Epistem so that near neighbors could be identified in environmental samples to distinguish from false positives. The expected melting temperatures of amplicons from the near neighbors were provided.

The choice of a pPCP1 plasmid target for *Y. pestis* detection is expected to be more sensitive than a chromosomal target, given the known higher target copy number present. This is reflected in the data presented, which is more than ten-fold more sensitive than the *B. anthracis* chromosomal assay. The tradeoff is specificity, as plasmids (including pPCP1) are often shared among different species in the same genus as well as other bacteria in a common community.

It would appear that there is substantial room for improvement in the bacterial assays, given the sensitivity is, at best, 100 GE per reaction. In this case, it reflects 10^3 or more target copies for pPCP1 per reaction and is significantly less sensitive than the theoretical single copy LOD. It is not known whether the general lack of sensitivity compared with other systems is due to poor assay performance or is a limitation of the amplification and detection system. With a wider set of better-designed assays, the flexibility of the system could be utilized in either "screening" (many singleplex assays for multiple organismal targets) or "confirmation" (multilocus testing for a single organism) modes. This would allow for rapid threat discrimination with high-confidence results in the field.

Assay Sensitivity

Assay sensitivity was as high as 100-fold less than the company's claimed 10 copies cloned into a plasmid vector. This lack of sensitivity is compounded by the relatively small template input volumes, driving up the *concentration* of the sensitivity LOD up to 10^5-10^7 copies per mL. Using template to rehydrate the lyophilized products could increase the sensitivity of concentration by 20-fold, but would not be expected to increase the overall sensitivity measured by genomics equivalents per reaction.

The novel Botulinum toxin assay designed for Epistem by SRC produced sensitivity equivalent to that of a standard sandwich immunoassay. The assay functions by the specific cleavage of a proprietary synthetic fluorophore-tagged peptide that when cleaved results in fluorescence transfer. The Genedrive was the only PCR platform able to provide direct toxin detection. Additional software development is needed in order to provide objective automated assay calls, which for this study were made by the operator using visual inspection of fluorescent curves.

The Genedrive system represents a portable and point of care molecular detection system. In addition, three separate reaction chambers give the user the option of either running three different reactions in parallel to provide more agent coverage, or running identical reactions in triplicate to provide higher confidence. Running assays in this manner precludes the opportunity of including controls for the assay.

At the time of this evaluation, the system could not be considered COTS technology, but rather a late beta test. There were significant issues with routine functions, such as pre-programmed RT-PCR assays and the lack of integrated battery power. The provided punch card sample preparation system was virtually non-functional and not able to provide an amplification-ready template. This significantly reduces the advantages of the portability of the system, which still requires additional infrastructure for template preparation.

Liat™

by IQuum

System Description

The Liat is an automated sample-to-result detection analyzer. IQuum's lab-in-a-tube (Liat) was designed to enable non-specialized personnel to perform "moderate complexity" tests in hospital labs or other near-patient setting. The Liat has automated sample processing in a flexible tube containing pre-packaged reagents. Peristaltic manipulations by actuators in the analyzer move the sample through each stage of sample processing ending with a PCR or RT-PCR amplification the sample for target identification. Currently IQuum has a FDA 510(K) cleared Liat Influenza A/B assay on the market which is intended for use in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA). For this testing IQuum developed two duplex assays, one that detects *Bacillus anthracis* and *Yersinia pestis* and another to detect Vaccinia and Venezuelan Equine Encephalitis.

System Specifications

Vendor: IQuum

Website: www.iquum.com

System Cost: $25,000.00

Assay Cost: Price on Request (Estimated to be $60 per test)

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: 2

Assay Shelf Life: 1 year at 4 °C

Sample Size Required: 200 µL

Type of Detection: Real-time nucleic acid amplification and fluorescence detection

Time to Result: 30 minutes

System Weight: 8.3 lbs

Operating Range: 40 - 122 °F (4 - 50 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab and field.

Test Bed Review

A combination of small footprint, automated sample prep, 20 minute run and sensitivity down to the single digits (CFU/mL), the Liat analyzer was a top performer both in the lab and in the field. Achieving detection levels down to 6.5 CFU/mL in their *Yersinia pestis* duplex assay, the Liat's sensitivity was superior to most other PCR platforms in the test bed. In addition to performing well in the presence of one agent, IQuum also scored well in the multiplex category, showing little to no loss of signal when samples were combined in a duplex format. During testing, the Liat did present with several error messages that required IQuum's intervention. However, these errors were a result of system checks put in place as a requirement for a FDA approved system.

In the field, the end-users were impressed with the ease of set-up and minimum amount of training required. One operator stated that "This device is the easiest piece of equipment I've ever used." Admittedly the current configuration of this system was not intended for outdoor use and end-users would like to see some modifications to its current design. The size of the buttons and the use of the stylus were top on their list. One user commented "The Login/Pin requirement is overkill for military applications and the stylus would get lost".

As a result of testing in an analytical and mobile laboratory and the field, the Liat showed a great deal of potential. We believe with little investment in its design (i.e., ruggedization of the exterior, integrated battery) this system would be a good fit in most, if not all, military testing applications.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX

Agent	Vendor Claimed LOD	Validation of Claimed LOD	Actual LOD
Bacillus anthracis	$2.00x10^{1}$	✗	$1.00x10^{3}$
Yersinia pestis	$5.00x10^{0}$	✓	$6.5x10^{0}$
Vaccinia	$2.00x10^{2}$	△	$2.50x10^{3}$
Venezuelan Equine Encephalitis	$4.00x10^{4}$	✓	$2.10x10^{3}$
Clostridium Botulinum Toxin	N/A	○	N/A

MULTIPLEX

Agent	Actual Singleplex LOD	Actual LOD Achieved	Multiplex LOD
Bacillus antracis	$1.00x10^{3}$	✓	$1.00x10^{3}$
Yersinia pestis	$5.00x10^{0}$	△	$1.00x10^{1}$
Vaccinia	$2.50x10^{3}$	△	$2.50x10^{4}$
Venezuelan Equine Encephalitis	$2.10x10^{3}$	△	$2.10x10^{4}$
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

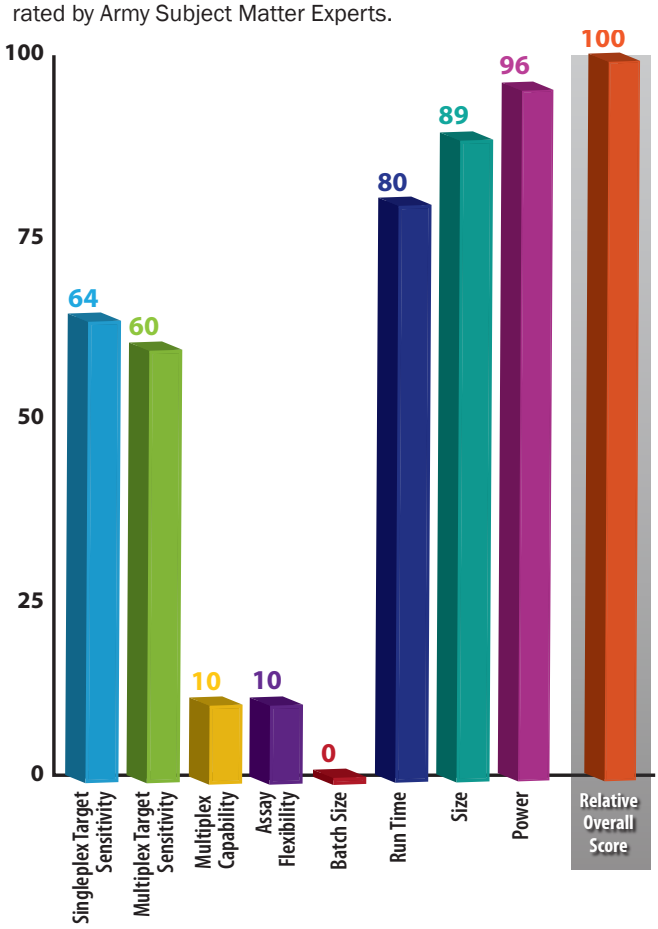
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

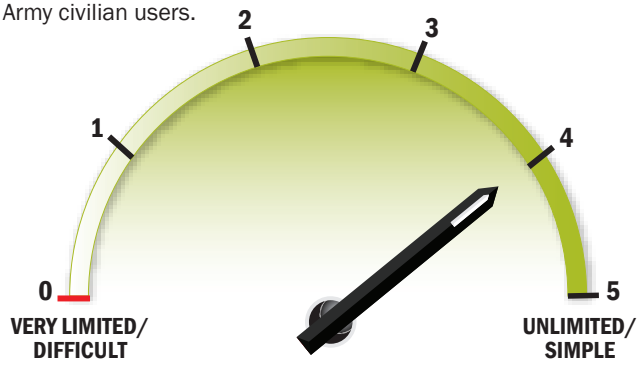
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



IQuum

Overview

IQuum's Liat is an automated sample-to-result detection analyzer. It was designed to enable non-specialized personnel to perform "moderate complexity" tests in hospital laboratories or other near-patient setting. The Liat has automated sample processing in a flexible tube containing pre-packaged reagents. Peristaltic manipulations by actuators in the analyzer move the sample through each stage of sample processing ending with a PCR or RT-PCR amplification the sample for target identification. Currently IQuum has an FDA 510(k) cleared Liat Influenza A/B assay on the market that is intended for use in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA). For this testing IQuum developed two duplex assays, one that detects *Bacillus anthracis* and *Yersinia pestis* and another to detect Vaccinia and Venezuelan Equine Encephalitis.

The Liat system was tested to evaluate the technology and verify vendor claims of LOD for four biothreat agents (two bacterial and two viral) using custom designed *B. anthracis/Y. pestis* and VEE/VAC assays. The Liat utilizes simple, load-and-go format assays in which the user adds a sample, inserts the tube into the analyzer, and walks away. The system uses actuators and peristaltic motion to move the sample to different regions of the assay tube where the nucleic acid is purified then amplified. Sample injection into the cassette takes seconds. The run-time of the *B. anthracis*/*Y. pestis* assay is 19 minutes while the run-time of the VEE/VAC assay is 32 minutes. The time discrepancy between the assays is due to the inclusion of a reverse transcription step in the VEE/VAC assay. The Liat is designed to be used in an FDA regulated laboratory environment; therefore, several of its features were considered hindrances for field or mobile laboratory use. Two specific examples are its narrow temperature operating range and requirement that users log-in with a username and PIN.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Gamma-irradiated *Bacillus anthracis* spores were diluted to $1x10^1$, $1x10^2$, and $1x10^3$ CFU/mL in PBS then tested, in triplicate, using the *B. anthracis/Y. pestis* duplex assay. Each 200µL sample was transferred directly into the tube and the tube was inserted immediately into the device. The vendor-stated LOD of this assay is $2x10^1$ CFU/mL, although 100 times more sample was required for consistent detection in this study. When testing began, two samples were tested at 10 CFU/mL and both were detected by the Liat device. Complete testing was not performed until two and a half months later. By that time sensitivity of the assays had diminished by a factor of 100. During that time, the operator also received invalid PCR result errors during the testing. An "invalid" PCR result is reported when either the Crossing Threshold (C_T) of the internal control is outside specification or the shape of the real-time data does not fit acceptance criteria. Issues of assay stability may be responsible for the decrease in performance. Final LOD of the *B. anthracis* assay was determined to be $1.0x10^3$ CFU/ml.

Table 20. *Bacillus anthracis* LOD

Concentration (Colony Forming Units (CFU)/mL)	Total CFU	Total Genome Equivalents (GE)	Results by Loci (Positives/Total Runs)
			Lethal Factor
$1.00x10^{1}$*	$2.00x10^{0}$	$4.76x10^{0}$	0/1
$1.00x10^{2}$	$2.00x10^{1}$	$4.76x10^{1}$	0/1
$1.00x10^{3}$**	**$2.00x10^{2}$**	**$4.76x10^{2}$**	**3/4**

* First tests were positive for 2/2 samples at 10 CFU, may indicate reagent stability issue
** Data includes "invalid" PCR result
Vendor Claimed LOD: $2.00x10^{1}$ CFU/mL

Yersinia pestis

Gram negative, rod-shaped bacterium

Gamma-irradiated *Yersinia pestis* cells were diluted to $6.5x10^{0}$, $6.5x10^{1}$, and $1.3x10^{2}$ CFU/mL in PBS then tested, in triplicate, using the *B. anthracis*/*Y. pestis* duplex assay. Each 200µL sample was transferred directly into the tube and the tube was inserted immediately into the device. The vendor stated LOD of this assay is $5x10^{0}$ CFU/mL, and this LOD was confirmed in this study.

Table 21. *Yersinia pestis* LOD

Concentration (CFU/mL)	Total CFU	Total GE	Results by Loci (Positives/Total Runs)
			Pesticin
$6.50x10^{0}$	**$1.30x10^{0}$**	**$1.80x10^{0}$**	**3/3**
$6.50x10^{1}$	$1.30x10^{1}$	$1.80x10^{1}$	3/3
$1.30x10^{2}$	$2.60x10^{1}$	$3.60x10^{1}$	3/3

Vendor Claimed LOD: $5.00x10^{0}$ CFU/mL

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Gamma-irradiated VAC was diluted to $2.5x10^{2}$ and $2.5x10^{3}$ PFU/mL in PBS then tested, in triplicate, using the VAC/VEE duplex assay. Each 200µL sample was transferred directly into the tube and the tube was inserted immediately into the device. The vendor stated LOD of this assay is 250 PFU/mL, although 10 times more sample was required for consistent detection in this study. One of the $2.50x10^{2}$ PFU/mL samples was reported invalid. An "invalid" result is reported when either the C_T of the internal control is outside specification or the shape of the real-time data does not fit acceptance criteria. The LOD of this assay was determined to be $2.50x10^{3}$ PFU/mL.

Table 22.Vaccinia LOD

Concentration (Plaque Forming Units (PFU)/mL)	Total PFU	Total GE	Results by Loci (Positives/Total Runs)
			Pesticin
$2.50x10^{2}$*	$5.00x10^{1}$	$7.21x10^{1}$	1/3
$2.50x10^{3}$	**$5.00x10^{2}$**	**$7.21x10^{2}$**	**3/3**

*Includes an "invalid" PCR result
Vendor Claimed LOD: $2.00x10^{2}$ PFU/mL

Venezuelan Equine Encephalitis

+ sense ssRNA, Alphavirus

Gamma-irradiated VEE viral particles were diluted to $2.1x10^2$, $2.1x10^3$, and $2.1x10^4$ PFU/mL in PBS then tested, in triplicate, using the VAC/VEE duplex assay. Each 200µL sample was transferred directly into the tube and the tube was inserted immediately into the device. The vendor stated LOD of this assay is $4.00x10^4$ PFU/mL. This evaluation found that the LOD was 20-fold better than reported by the manufacturer. The LOD of this assay was determined to be $2.10x10^3$ PFU/mL.

Table 23. Venezuelan Equine Encephalitis LOD

Concentration (PFU/mL)	Total PFU	Total GE	Results by Loci (Positives/Total Runs)
			Pesticin
$2.10x10^2$	$4.20x10^1$	$4.20x10^1$	0/3
$2.10x10^3$	**$4.20x10^2$**	**$4.20x10^2$**	**3/3**
$2.10x10^4$	$4.20x10^3$	$4.20x10^3$	3/3

*VEE provider did not quantitate a GE to PFU ratio. A ratio of one was used for this study.
Vendor Claimed LOD: $4.00x10^4$ PFU/mL

Clostridium botulinum Type A toxin

Protein toxin

No assay was developed for *C. botulinum* toxin or DNA.

Multiplex Detection

Gamma-irradiated *B. anthracis* spores and *Y. pestis* cells were each diluted into PBS then mixed to create samples with 1,000 CFU/mL *B. anthracis* and 6.5 CFU/mL *Y. pestis*. The first mixed sample was tested and both agents were detected. In the second run, *Y. pestis* was not detected. In accordance with established protocols, fresh samples were created with 1,000 CFU/mL *B. anthracis* and 65 CFU/mL *Y. pestis*. This sample, with 10 times more *Y. pestis* was tested in triplicate. Both agents were detected in all three replicates.

Gamma-irradiated VAC and VEE viral particles were each diluted into PBS then mixed to create samples with 2,500 PFU/mL VAC and 2,100 PFU/mL VEE and tested in triplicate with the VAC/VEE duplex assay. VEE was not detected in any of the three assays; therefore fresh samples containing 10-fold more VEE were created and tested in triplicate. In these replicates, VEE was detected in all three tests while VAC was only detected in two of the three assays. Again, the concentration of the agent not yielding consistent detection, in this case VAC, was increased 10-fold. The samples, now containing 25,000 PFU/mL VAC and 21,000 PFU/mL VEE, were tested in triplicate. Both targets were detected in all three assays.

Table 24. Multiplex Evaluation

Agent	Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
B. anthracis	**$1.00x10^3$**	**$2.00x10^2$**	**$4.76x10^2$**	**5/5**
Y. pestis	$6.50x10^0$	$1.30x10^0$	$1.77x10^0$	1/2
	$6.50x10^1$	**$1.30x10^1$**	**$1.77x10^1$**	**3/3**
Agent	**Concentration (PFU/mL)**	**Total PFU**	**Total EG**	**Results (Positives/Total Runs)**
VAC	$2.50x10^3$	$5.00x10^2$	$7.20x10^2$	5/6
	$2.50x10^4$	**$5.00x10^3$**	**$7.20x10^3$**	**3/3**
VEE	$2.10x10^3$	$4.20x10^2$	$4.20x10^2$	2/3
	$2.10x10^4$	**$4.20x10^3$**	**$4.20x10^3$**	**6/6**

*Provider of VEE did not quantitate a GE to PFU ratio. For the purpose of this study a ratio of 1 was used.

Discussion

Call Assignments

All individual system calls are made for each individual organism from a single locus PCR amplification. The tested format was duplex assays pairing *B. anthracis* and *Y. pestis* or VEE and VAC, each of which includes an internal positive amplification control.

Each PCR amplification curve is processed with an algorithm to determine C_T values and whether end-point PCR fluorescent values are within an acceptable range, which determines whether a given call is "positive" or "negative." Additional curve fitness parameters are also evaluated to insure that the amplification fits an acceptable logarithmic profile. Any positive result based upon fluorescence measurements but failing the curve profile metrics is called as "indeterminate." If all three PCR profiles (two target organisms and internal control) fail the curve fitness determination, the entire assay is called as "invalid." Finally, the internal control must be determined to be positive in order to make a "negative" call for both target organisms: otherwise an "invalid" assay call is made. However, a positive result with either of the target organisms may inhibit the amplification of the internal control target, presumably due to consumption of deoxyribonucleotide triphosphates and monopolization of polymerase. Therefore, any positive result from one of the three PCR reactions with appropriate curve metrics is considered a positive amplification control. While this is certainly an acceptable theoretical assumption, perhaps a better theoretical solution (particularly for any assay undergoing rigorous review, such as an FDA submission for clinical applications) would be to develop a more rigorous internal amplification control.

There were multiple ambiguous PCR calls made that did not specifically correlate with template concentrations near the LOD. The nature of these calls is not known, but potential sources of this error may include:

- Systemic errors during software analysis of raw data
- Reagent instability or variability
- Intermittent hardware fault of the specific instrument used for this evaluation
- Hardware design flaw inherent to the manufactured product

These issues will need to be addressed in any acquisition program in order to ensure robust and consistent performance, especially in challenging environments outside of a controlled laboratory setting.

Plasmid and Chromosomal Targets

The single locus detections scheme employed in the two assays does not allow for multiplex detection of both chromosomal and multiple plasmid PCR targets routinely used for high confidence and simultaneous detection of the threat agents and virulence islands required for pathogenesis. Presumably, the more highly multiplex formats claimed but not demonstrated (16-plex based upon independent and spectrally separated excitation and emission channels) and parallel processing could produce the required data density for this level of sophisticated and conclusive multi-agent detection. However, this capability was not tested in this laboratory evaluation.

For both of the bacterial targets, the PCR loci were located on plasmids (Lethal Factor on pXO1 for *B. anthracis* and Pesticin on pPCP-1 for *Y. pestis*). While plasmid targets tend to be present in higher concentrations than chromosomal markers, making them attractive for sensitive detection, both have been known to be to be present in other members on the same genus. Intact or partial pXO1 is often seen in other pathogenic group I *Bacilli* such as *B. cereus*. In the case of Pesticin, it is not only found in other *Yersinia* but closely related homologs are seen widely throughout the gram-negative enterics. All of these potentially cross-reacting organisms also cause human disease and are common in environmental samples. For the purposes of the technical evaluation of equipment in this study, this is not a pertinent issue.

Assay Sensitivity

Assay sensitivity has been demonstrated to be as low as <10 CFU with 100% detection. The excellent sensitivity is in part due to the robust sample preparation, which includes a relatively large input sample volume. This entire nucleic acid preparation is included into a single multiplexed PCR reaction rather than having to parse the purified sample into multiple parallel reactions. This is a significant advantage in terms of sensitivity, especially when surveying for multiple target organisms.

However, there were a couple of observed limitations to the existing configuration that indicated that optimal sensitivity was not achieved. First, sensitivity between instrument runs varied as widely as 100-fold, which was attributed to reagent instability and/or inconsistency. Second, only very low levels of agent and loci multiplexing were tested. Even at this level, there was evidence of unbalanced competing PCR reactions, 10-100 fold variability in sensitivity of DNA targets, and 10-fold loss of sensitivity in multiplex format. Finally, deeper multiplex assays were not evaluated and remain an unknown capability in terms of sensitivity.

T-COR 4™

by Tetracore, Inc.

System Description

The T-COR 4 is a portable Real-Time PCR thermocycler with four independent sample wells capable of 2 color detection. The T-COR 4 is a field deployable battery powered system, but lacks sample preparation capabilities. Tetracore's real-time PCR reagents are stored at room temperature with 20+ assays currently available. The system in encased in a heavy duty protective rubber sleeve with an internal 8 hour battery for the thermal cycler. For this testing, Tetracore's *Bacillus anthracis* pXO1 assay and *Yersinia pestis* assay were evaluated. Each assay contains reagents for specific target detection using the FAM fluorophore and an internal control detected with CY5 fluorophore.

System Specifications

Vendor: Tetracore, Inc.

Website: www.tetracore.com

System Cost: $38,500.00

Assay Cost: $16,000.00

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: One. Multiple formats available

Assay Shelf Life: 1 year at room temperature

Sample Size Required: 3 µL

Type of Detection: Nucleic Acid

Time to Result: 45 minutes

System Weight: 6.2 lbs (w/o required centrifuge)

Operating Range: 39–113 °F (4–50 °C)

Test Bed Review

The T-COR 4 is small, lightweight and battery operated, making it appealing for the field. The main shortcomings were the number of targets (1) per test and lack of integrated sample preparation. With purified samples, the system scores high in the laboratory, sensitivities in the femtogram range. The 20 minute runtime and real time viewing are appealing, unfortunately without on board sample preparation, the scores are lower than the other systems. Additionally, Tetracore was only able to provide 2 of the 5 requested targets, with no ability to design new assays for this testing. The test results could not be saved on the device, as configured. Tetracore can provide software to operate the instrument, and save and analyze data using an external computer.

In the field, the end-users liked the ease-of-use of the system and had no trouble running this system independently. Although one soldier commented, "It doesn't really add anything additional", another added, "It's quick to start up and easy to use". End users liked that the results were easy to view and interpret. Consistent results were an additional plus of this system. Training time was minimal and the consumables were easy to handle in MOPP gear. The small centrifuge for the PCR tubes added to the footprint for field operators.

As a result of testing in an analytical and mobile laboratory and the field the T-COR 4 was a consistent and easy to use system. The lack of onboard sample preparation is a negative, but the system is a reliable workhorse.

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab and field.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = fg/uL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	No Claim	○	$3.2x10^{-1}$
Yersinia pestis	No Claim	○	$1.3x10^{1}$
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$3.2x10^{-1}$	○	N/A
Yersinia pestis	$1.3x10^{1}$	○	N/A
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

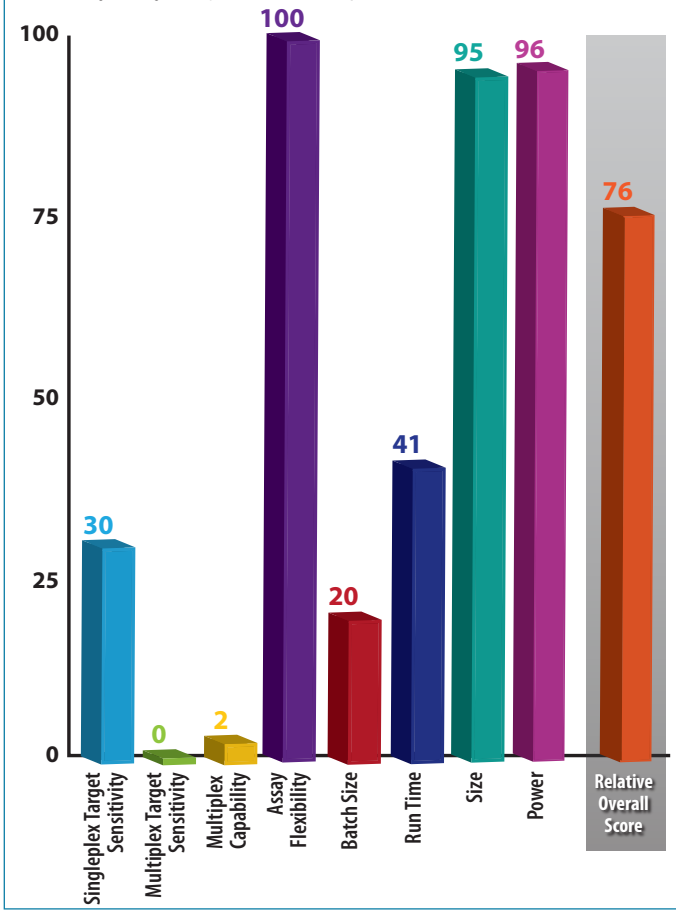
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

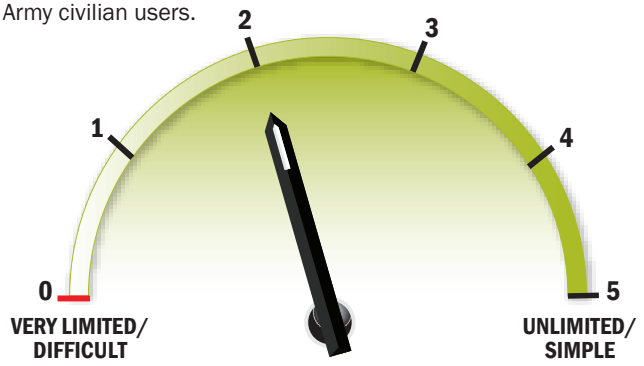
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Tetracore®

Overview

The T-COR 4 is a portable Real-Time PCR thermocycler with four independent sample wells capable of two-color detection. The T-COR 4 is a field deployable battery powered system, but lacks sample preparation capabilities. Tetracore's real-time PCR reagents are stored at room temperature with more than 20 assays currently available. The system in encased in a heavy duty protective rubber sleeve with an internal eight hour battery for the thermal cycler. For this testing, Tetracore's *B. anthracis* pXO1 assay and *Y. pestis* assay were evaluated. Each assay contains reagents for specific target detection using the FAM fluorophore and an internal control detected with CY5 fluorophore.

The Tetracore® T-COR 4 platform running the *B. anthracis* pOX-1 or *Y. pestis* assay was tested to evaluate the technology and verify vendor claims of LOD for two bacterial biothreat agents (*B. anthracis* and *Y. pestis*). The Tetracore T-COR 4 *Clostridium botulinum* A/B assay was also utilized in this evaluation to determine whether the system could detect residual BoNT A DNA in a preparation of holotoxin.

The T-COR 4 contains a photomultiplier and filters to monitor two emission wavelengths simultaneously. The presence of target is monitored on the FAM channel while the Cy5 channel is reserved for the amplification of an internal control preloaded and lyophilized in the assay tubes. Thermal cycling was completed in about 45 minutes using the "PCR" algorithm common to both assays evaluated in this study.

Unlike the majority of systems tested in this evaluation, the T-COR 4 does not have integrated sample preparation or nucleic acid extraction. Therefore, purified DNA rather than whole, inactivated agent, was used in the assessment of the device. The T-COR 4 is simple to set up and operate and consists of a base unit capable of analyzing up to four samples or controls simultaneously and a separate mini-centrifuge with custom rotor. Both the base unit and mini-centrifuge are powered by integrated, rechargeable batteries. Each kit is supplied with four assay tubes containing lyophilized reagents within a sealed package. To analyze a sample on the device, the user rehydrates tubes with 27µL water, adds 3µL sample or control, closes the tubes, and mixes the reactants. Just prior to loading the tubes into the device, the user spins them briefly with the mini-centrifuge to collect the reaction components in the bottom of the tube. The user selects the desired program from a list of loaded assays using the four hard keys on the device and selects start. Results are viewable in real-time as a fluorescence versus cycle graph. Additionally, a table displaying C_T values can be viewed.

No multiplexing was performed during this evaluation, as the system is comprised of four two-color real time PCR modules for a single analyte detection and internal positive control per module. As an alternative to multiplex assays, a user can run four separate assays simultaneously as long as each assay requires identical thermal cycling parameters. All testing was performed with DNA purified from bacterial reference materials using the Qiagen® QIAamp® DNA Mini Kit.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Purified DNA extracted from gamma-irradiated *Bacillus anthracis* spores was serially diluted in 10-fold increments into water and evaluated on the T-COR 4 platform with the *B. anthracis* pOX-1 assay. Each dilution was tested in triplicate until a dilution was determined to yield inconsistent or no detection of *B. anthracis*. SmartCycler tubes containing lyophilized reagents were rehydrated with 27µL water then 3µL sample was added. As directed by kit instructions, samples were analyzed using the preinstalled PCR Program algorithm. The LOD of the device was determined to be $2.38x10^{1}$ CFU/mL.

Table 25. *Bacillus anthracis* LOD

Concentration (femtograms (fg)/µL)	Total fg	Total Genome Equivalents (GE)	Equivalent CFU/mL	Results by Loci (Positives/Total Runs)
				pX01
$3.20x10^{-1}$	**$9.60\ x10^{-1}$**	**$1.70x10^{-1}$**	**$2.38x10^{1}$**	**3/3**
$3.20x10^{0}$	$9.60\ x10^{0}$	$1.70x10^{0}$	$2.38x10^{2}$	3/3
$3.20x10^{1}$	$9.60\ x10^{1}$	$1.70x10^{1}$	$2.38x10^{3}$	3/3

Vendor Claimed LOD: N/A

Yersinia pestis

Gram negative, rod-shaped bacterium

Purified DNA extracted from gamma-irradiated *Yersinia pestis* cells was serially diluted in 10-fold increments into water and evaluated on the T-COR 4 platform with the *Y. pestis* assay. The actual target of the assay is not stated by Tetracore. Each dilution was tested in triplicate until a dilution was determined to yield inconsistent or no detection of *B. anthracis*. SmartCycler tubes containing lyophilized reagents were rehydrated with 27µL water then 3µL sample was added. As directed by kit instructions, samples were analyzed using the preinstalled PCR program algorithm. The LOD of the device was determined to be $1.80x10^{3}$ CFU/mL.

Table 26. *Yersinia pestis* LOD

Concentration (fg/µL)	Total fg	Total GE	Equivalent CFU/mL	Results by Loci (Positives/Total Runs)
				Yersinia murine toxin (ymt)
$1.30x10^{0}$	$3.91x10^{0}$	$7.51x10^{-1}$	$1.80x10^{2}$	2/3
$1.30x10^{1}$	**$3.91x10^{1}$**	**$7.51x10^{0}$**	**$1.80x10^{3}$**	**3/3**
$1.30x10^{2}$	$3.91x10^{2}$	$7.51x10^{1}$	$1.80x10^{4}$	3/3

Vendor Claimed LOD: N/A

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

No Assay Available

Venezuelan Equine Encephalitis
+ sense ssRNA, Alphavirus

No Assay Available

Clostridium botulinum Type A toxin
Protein toxin

Tetracore supplies a *C. botulinum* A/B assay for the T-COR 4. Although the target is DNA rather than the active toxin used in this assessment, the assay was evaluated for its ability to detect residual host DNA in the toxin preparation. BoNT A, as supplied by Metabiologics, is produced in its native organism; therefore, the toxin preparation was tested to determine if it contained detectable *C. botulinum* DNA. Samples were tested in duplicate along with a single replicate each of a positive and negative control. One of the two replicates at 1,000ng/mL was reported as positive for *C. botulinum* DNA, but the form of the "fluorescence versus cycle" plot was not shaped as expected for typical qPCR data. Therefore, this positive detection was discounted. Lack of detectable levels of *C. botulinum* DNA in the toxin preparation is consistent with information obtained from Metabiologics that the product had very low A_{260} measurement, indicative of no host DNA.

Table 27. *Clostridium botulinum* toxin Limit of Detection LOD

Concentration (nanograms (ng)/mL)	Total Toxin (ng)	Instrument Call	Results by Loci (Positives/Total Runs)
			Unknown
$1.00x10^{2}$	$3.00x10^{-1}$	0/2	0/2
$1.00x10^{3}$[t]	$3.00x10^{0}$	1/2	1/2

[t] Positive sample had a very low C_T (12.55) and abnormal fluorescence versus cycle curve shape
Vendor Claimed LOD: N/A

Discussion

Call Assignments
Call assignments are made based upon two-color real-time PCR reactions. The internal positive control assures amplification and lack of significant PCR inhibition. The control was successful both in no template controls and all concentrations of analytes tested. The second color channel indicates signal from the target analyte amplification. A proprietary algorithm provides analysis of the exponential signal curve, providing a "Smart C_T" value and evaluating curve fitness. Results are either "pos" or "neg" with a given C_T value if present. A "neg" result requires a positive internal control result to rule out a false negative reaction.

Plasmid and Chromosomal Targets
The *B. anthracis* assay tested a PCR target on the pXO1 plasmid, which is usually present in multiple copies. This has both advantages and disadvantages as a detection strategy, as higher copy number allows for higher confidence detection and lower LOD. However, relying on a single plasmid for positive detection of *B. anthracis* is known to have significant specificity problems as other members (both pathogenic and non-pathogenic) of closely related members of the Group I *Bacilli*, and especially *B. cereus*, often share partial or entire plasmid sequences in pXO1 and pXO2. This approach of assay choice and instrument configuration has advantages such as the ability to determine extent of contamination following a known release of agent, but would require high-confidence confirmation before taking high-consequence actions in response to a positive result.

The target of the *Y. pestis* assay is *ymt* on pMT1 and the sensitivity is on close-order to the results of the low-copy plasmid targets. The small, high-copy pPCP1 plasmid would be expected to more sensitive. Any advantage here would be more obvious when looking at sampling efficiency and the ability to detect trace amounts of larger samples (e.g., swabs) following sample processing into a small volume of template.

Assay Sensitivity

Unlike the majority of other systems tested in this study, the T-COR 4 system required purification of the DNA prior to sample analysis. As such the LOD determined in this study was extrapolated using the quality control metrics supplied by the vendor and an approximate genome size to normalize the results and adjust the units for easier comparison to other devices. The values reported here do not take in to account any inefficiencies in the DNA extraction procedure nor correct for variability in copy number of the plasmid targets.

Assay sensitivity was demonstrated to be as low as 24 CFU/mL with 100% detection of *B. anthracis* which is excellent considering the sample input is only 3µL of purified DNA. Sensitivity of the *Y. pestis* assay was approximately 10-fold higher reflecting variability in target sensitivity.

Like the other PCR systems in this study, the T-COR 4 was unable to reliably detect trace amounts of *Botulinum* DNA in the toxin preparation. One replicate was reported as positive although curve shape was not indicative of a true amplification. This result highlights a limitation of the device – the lack of curve fit metrics for determining C_Ts.

NIDS®

by ANP Technologies

System Specifications

Vendor: ANP Technologies

Website: www.anptinc.com

System Cost: $6,500.00

Assay Cost: $45.00

Assay Storage Requirements: Room Temperature

Agents Tested per Assay: 5 per Assay, Multiple formats available

Assay Shelf Life: 2 years from receipt at room temperature

Sample Size Required: 100–200 µL

Type of Detection: Antibody

Time to Result: 15 minutes

System Weight: 1.6 lbs

Operating Range: 40–122 °F (4–50 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). NIDS assay used with Stand Alone Reader had successful operation in Mobile lab and field.

System Description

ANP has developed the Nano Intelligent Detection System, or NIDS. The NIDS is a multiplexed Handheld Assay (HHA) together with a palm-sized, portable, ruggedized optical Stand Alone Reader (SAR III). The NIDS technology uses an antibody nanomanipulation technique that orients each antibody so that there are optimal biosensing regions available for antigen binding and sandwich formation. Because of this nanomanipulation, the NIDS is the first HHA that claims to have no "Hook Effect". The NIDS was designed to take all of the guess work out of interpreting HHA results by different responders in field conditions with poor lighting and limited visibility while wearing personal protective equipment.

Test Bed Review

The NIDS is small, rapid and has the ability to detect bacterial, viral, and protein toxins. However, it still delivers below average sensitivity and high false positive rates that we've come to expect with a typical HHA. Detection levels ranging from $1x10^6$ to $1x10^8$ CFU/mL for a singleplex assay and $1x10^6$ to $1x10^9$ CFU/mL in the multiplex, gave the NIDS an overall low score in the Target Identification Category of the Criteria Table.

Where the NIDS did fair well is ease of use and small size. Little to no sample preparation time is required for this system, maintenance time is low and the overall sample to result is 15 minutes. These attributes yielded scores of 70-100% in 4 of the 5 categories.

In the field, the end-users were impressed with the ease of set-up and minimum amount of training required. However one operator, familiar with typical HHAs remarked "The reader makes it easier than current HHAs, but it's still an HHA." In addition, one user experienced false positives while running the device, which was quickly fixed after cleaning the lens. However this result did lead to concerns of contamination. On a positive note, one soldier commented, "The device is small enough to fit into the pouch of my body armor".

As a result of testing in an analytical and mobile laboratory and in the ECBC Skippers Point Site the NIDS overall performance was mediocre at best. As one of the only currently fielded systems in our test bed, better results were expected from this system and users were disappointed by the recurring false positives. Clearly there is a need for a more sensitive and accurate identification of biothreats

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	$1.00x10^{6}$	⚠	$1.00x10^{7}$
Yersinia pestis	$2.50x10^{5}$	⚠	$2.50x10^{6}$
Vaccinia	$1.00x10^{6}$	✖	$>1.00x10^{8}$
Venezuelan Equine Encephalitis	$1.00x10^{8}$	⚠	$1.00x10^{9}$
Clostridium Botulinum Toxin	$5.00x10^{1}$	✔	$5.00x10^{1}$
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$1.00x10^{7}$	✔	$1.00x10^{7}$
Yersinia pestis	$1.00x10^{6}$	✔	$1.00x10^{6}$
Vaccinia	$>1.00x10^{8}$	✔	$1.00x10^{8}$
Venezuelan Equine Encephalitis	$1.00x10^{9}$	✔	$1.00x10^{9}$
Clostridium Botulinum Toxin	$5.00x10^{1}$	⚠	$5.00x10^{2}$

✔ Validated ⚠ Not Validated (≤1 log difference) ✖ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

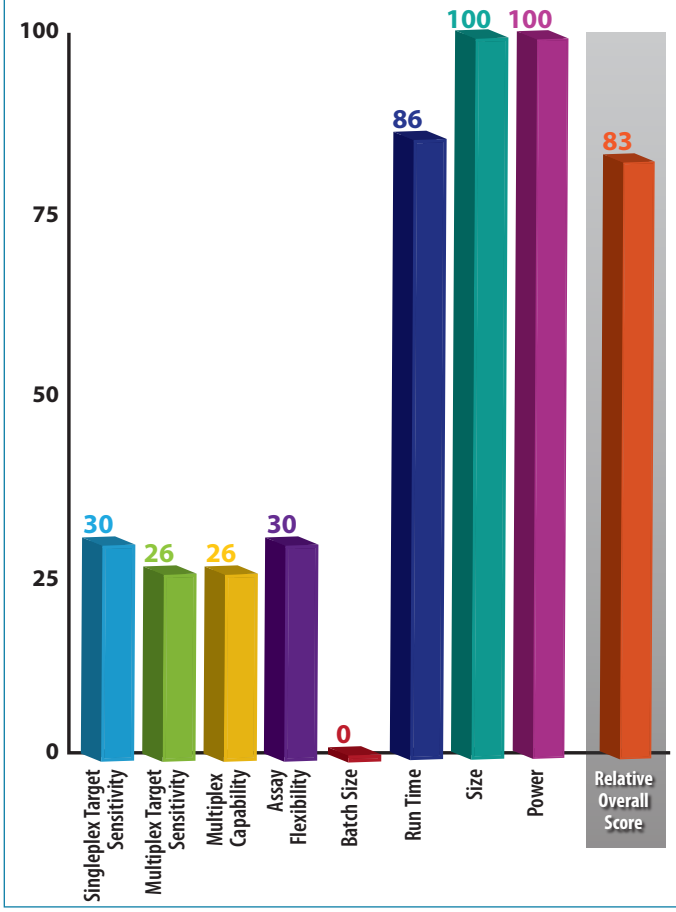
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

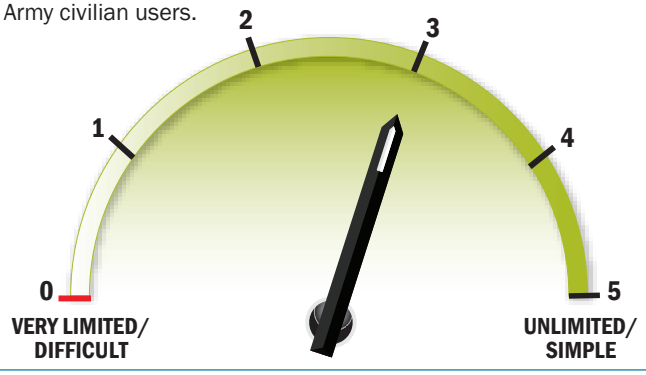
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Overview

ANP Technologies' Nano Intelligent Detection System (NIDS) is a multiplexed Handheld Assay (HHA) together with a palm-sized, portable, ruggedized optical Stand Alone Reader (SAR III). The NIDS technology uses an antibody nanomanipulation technique that orients each antibody so that there are optimal biosensing regions available for antigen binding and sandwich formation. Because of this nanomanipulation, the NIDS is the first HHA that claims to have no "hook effect," which causes false negative results at high target concentrations The NIDS was designed to take the guess work and inconsistency out of interpreting HHA results that is sometimes experienced by different end-users wearing personal protective equipment in field conditions with poor visibility.

NIDS assays are targeted against multiple agents – up to 5 – in the same assay, in contrast to other hand-held lateral flow assays that have single targets per assay cartridge. By reducing the "hook effect," the NIDS design also reduces the need to run serial dilutions to obtain reliable results. The NIDS assays offer the ability to directly detect proteins from bacteria, viruses, or protein-based toxins and to provide an orthogonal confirmatory technology to nucleic acid-based systems. Other desirable characteristics of this system are the low cost, small size, battery power, two year shelf-life, and simplicity of use. ANP has offered various NIDS multiplex assays for combinations of biological threat targets in 2-plex, 3-plex, 4-plex and 5-plex configurations.

The NIDS assays are simple to operate, requiring the user to directly add 100µL of agent to the sample pad. End-users have flexibility in adding samples to the NIDS via either a pipette or a dropper or other disposable device. After a 15 minute development time, the cartridges are inserted into the SAR III. With very few manipulations of the SAR III buttons, the detection data and an image of the biosensing region of the NIDS are stored for subsequent review. The SAR III is supplied with a microSD card that stores up to 300 cassette images with associated sample data. The SAR III can obtain power from either two double-A batteries, a USB power cord, or a direct 110 volt power cord.

The NIDS with SAR III system was tested using ANP's commercially available 5-plex Biothreat Assays to assess the technology and verify vendor claims of LOD for five biothreat agents (two bacterial, two viral, and one protein toxin). The "5-Plex 1" cartridge was designed to detect *Bacillus anthracis*, *Francisella tularensis,* Ricin, Staphylococcal enterotoxin B, and BoNT A. The "5-Plex 2" cartridge was designed to detect *Yersinia pestis*, Orthopox Vaccinia, Venezuelan Equine Encephalitis, *Coxiella Burnetii,* and *Brucella melitensis*. For this testing, *B. anthracis* and BoNT A were assessed using "5-Plex 1" and *Y. pestis*, VAC and VEE were evaluated using the "5-Plex 2" cartridges.

Data

Bacillus anthracis

Gram positive spore-forming bacteria

Gamma-irradiated *B. anthracis* spores were diluted to $1x10^5$, $1x10^6$, and $1x10^7$ CFU/mL in assay buffer (PBS, 0.1% Triton X-100, 0.04% Kathon® CG/ICP) supplied by ANP then tested on "5-Plex 1" cartridges. One hundred microliters of each sample was applied to the sample pad in triplicate assays and allowed

to develop for 15 minutes. The cartridges were imaged on the SAR III. Although the vendor claimed the LOD to be $1x10^6$ CFU/mL, 10-fold more was required for successful detection. The LOD of the assay was determined to be $1.00x10^7$ CFU/mL.

NOTE: A single false positive identification of *F. tularensis* occurred during assays of the lowest concentrated samples. However, the result could not be repeated by additional imaging of the cassette. Visual examination of the lens surface after obtaining the false positive found the glass surface to be clean and without smudges.

Table 28. *Bacillus anthracis* LOD

Concentration (Colony Forming Units (CFU)/mL)	Total CFU	Total Genome Equivalents (GE)	Results (Positives/Total Runs)
$1.00x10^5$*	$1.00x10^4$	$2.38x10^4$	0/3
$1.00x10^6$	$1.00x10^5$	$2.38x10^5$	0/3
$1.00x10^7$	**$1.00x10^6$**	**$2.38x10^6$**	**3/3**

* Single false positive result for *F. tularensis*
Vendor Claimed LOD: $1.00x10^6$ CFU/mL

Yersinia pestis

Gram negative bacteria

Gamma-irradiated *Y.pestis* was diluted to $2.5x10^4$, $2.5x10^5$, and $2.5x10^6$ CFU/mL in assay buffer supplied by ANP (PBS, 0.1% Triton X-100, 0.04% Kathon® CG/ICP) then tested on the "5-Plex 2" cartridge. One hundred microliters of each sample was applied to the sample pad in triplicate and allowed to develop for 15 minutes. After the development period, the cartridges were imaged on the reader. Although the vendor claimed LOD of the assay to be $2.5x10^5$ CFU/mL, 10-fold more *Y. pestis* was required for successful detection. The LOD of the assay was determined to be $2.50x10^6$ CFU/mL.

Table 29. *Yersinia pestis* LOD

Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
$2.5x10^4$	$2.5x10^3$	$3.46x10^3$	0/3
$2.5x10^5$	$2.5x10^4$	$3.46x10^4$	0/3
$2.5x10^6$	**$2.5x10^5$**	**$3.46x10^5$**	**3/3**

Vendor claimed LOD: $2.50x10^5$ CFU/mL

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Gamma-irradiated VAC virus was diluted to $1x10^4$, $1x10^5$, and $1x10^6$ PFU/mL in assay buffer supplied by ANP (PBS, 0.1% Triton X-100, 0.04% Kathon® CG/ICP) then tested on the "5-Plex 2" cartridge. One hundred microliters of each sample was applied to the sample pad in triplicate and allowed to develop for 15 minutes. After the development period, the cartridges were imaged on the reader. None of the nine samples tested resulted in a positive detection of VAC; therefore, a single sample at $1x10^8$ PFU/mL was tested and also found to be negative. Testing was repeated with samples diluted to $1x10^7$ and $1x10^8$ PFU/mL. In the repeated testing, a single replicate of the highest concentration tested yielded a positive result (see Table 30). Therefore, the LOD of the assay for VAC is greater than $1x10^8$ PFU/mL. Testing of

the antigen at higher concentration would have required the sample to be added without dilution; therefore, it was not completed.

Table 30. Vaccinia LOD

Concentration (PFU/mL)	PFU	Total GE	Results (Positives/Total Runs)
$1.00x10^5$	$1.00x10^4$	$1.44x10^4$	0/3
$1.00x10^6$	$1.00x10^5$	$1.44x10^5$	0/3
$1.00x10^7$	$1.00x10^6$	$1.44x10^6$	0/6
$1.00x10^8$	$1.00x10^7$	$1.44x10^7$	1/4

Vendor Claimed LOD: N/A

Venezuelan Equine Encephalitis

+ sense ssRNA, Alphavirus

Gamma-irradiated VEE virus was diluted to $1x10^8$ and $1x10^9$ PFU/mL in assay buffer supplied by ANP (PBS, 0.1% Triton X-100, 0.04% Kathon® CG/ICP) then tested on the "5-Plex 2" cartridge. One hundred microliters of each sample was applied to the sample pad in triplicate and allowed to develop for 15 minutes. Although the vendor claimed LOD of the assay to be $1x10^8$ PFU/mL, 10-fold more was required for successful detection in this study. The LOD of the assay was determined to be $1x10^9$ PFU/mL.

Table 31. Venezuelan Equine Encephalitis LOD

Concentration (PFU/mL)	Total PFU	Total GE	Results (Positives/Total Runs)
$1x10^8$	$1x10^7$	$1x10^7$	0/3
$1x10^9$	**$1x10^8$**	**$1x10^8$**	**3/3**

Vendor Claimed LOD $1.00x10^8$ PFU/mL

Clostridium botulinum Type A toxin

Protein toxin

BoNT A was diluted to five, 50, and 500ng/mL into PBS then tested on the "5-Plex 1" assay cartridge. One hundred microliters of each sample was applied to the sample pad in triplicate and allowed to develop for 15 minutes. After the development period, the cartridges were imaged. The vendor claimed LOD of the assay is 50ng/mL; this limit was confirmed during this testing.

Table 32. *Clostridium botulinum* Type A LOD

Concentration (ng/mL)	Total Toxin (ng)	Results (Positives/Total Runs)
$5x10^0$	$5.00x10^{-1}$	0/3
$5x10^1$	**$5.00x10^0$**	**3/3**
$5x10^2$	$5.00x10^1$	3/3

Vendor Claimed LOD: $5.00x10^1$ng/mL

Multiplex Testing

All five antigens used in this assessment can be detected by the NIDS cartridges, but the targets are spread over two cartridges, 5-Plex 1 and 5-Plex 2. For multiplex testing, samples were created containing only antigens that could be detected with the assay used. Triplicate samples containing antigens diluted to the concentration determined as LOD through empiric singleplex testing were

evaluated by applying 100µL to the appropriate cartridge. After the development period, the cartridges were imaged. The singleplex LOD for each assay was confirmed as valid for multiplex testing except BoNT A. The *B. anthracis*/BoNT A evaluation was repeated with the concentration of the toxin increased 10-fold. Both targets were detected in all three replicates upon repeat testing.

Table 33. 5-Plex 1 Multiplex Evaluation

Agent	Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
B. anthracis	**$1.00x10^{7}$***	**$1.00x10^{6}$**	**$2.38x10^{6}$**	**6/6**
Agent	**Concentration (ng/mL)**	**Total Toxin (ng)**		**Results (Positives/Total Runs)**
BoNT A	$5.00x10^{1}$	$5.00x10^{0}$		1/3
	$5.00x10^{2}$	**$5.00x10^{1}$**		**3/3**

Table 34. 5-Plex 2 Multiplex Evaluation

Agent	Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
Y. pestis	**$2.50x10_{6}$**	**$2.50x10^{5}$**	**$3.46x10^{5}$**	**3/3**
Agent	**Concentration (PFU/mL)**	**Total PFU**	**Total GE**	**Results (Positives/Total Runs)**
VAC	**$1.00x10^{8}$**	**$1.00x10^{7}$**	**$1.44x10^{7}$**	**3/3**
VEE	**$1.00x10^{9}$**	**$1.00x10^{8}$**	**$1.00x10^{8}$**	**3/3**

* Single false positive result for *F. tularensis*

Discussion

Call Assignment

Call assignments are made based upon SAR III optical scanning of five sectors of the NIDS assay cartridge. The use of the scanner and software band-calling software eliminates the subjectivity of manual result assignments, and is intended for consistent detection of faint results. However, the laboratory assessment discovered that the software occasionally called positive results where no band was apparent (i.e., false positive). Each cartridge includes an assay positive control to ensure fluidics, assay chemistry, and detection hardware is functioning properly. Data is displayed as "OK" (positive control only), red (positive), and green (negative) for each assay region. Unfortunately, the software makes a call by a region rather than assay target which may occasionally lead to confusion for the end user.

Assay Sensitivity

Assay sensitivity across bacterial, viral, and toxin targets was relatively poor, in comparison to nucleic acid-based detection systems and even other antibody-based systems. In most cases (except for the protein toxin), the LOD was one to two orders of magnitude less sensitive than vendor claims. For all organisms, the LOD was at or above the LD_{50} or ID_{50} of the organism or toxin. Some testing was limited by the availability of high enough concentrations of stock target solution. A major implication is that the NIDS may not have true utility for biological agent identification except in cases where gross contamination is present, the inference being that the system will produce false-negative results when only modest (but still dangerous) amounts of agent are present.

In singleplex testing, a single false-positive result was noted (*Francisella tularensis*) as positive when a small, undetectable amount of *B. anthracis* was tested. Rescanning of the assay cartridge with this false-

positive result was consistently positive but the result could not be recapitulated with any amount of *B. anthracis* antigen. Another false-positive *F. tularensis* result was seen in the multiplex *B. anthracis* and BoNT A testing, but the result was not seen with repeated scans of the same cartridge. Contamination with *F. tularensis* antigen is unlikely, given the high concentrations of materials that would be required to produce the result and the fact that this antigen was not in use in the laboratory at any time during instrumentation evaluation. It is possible that the assay itself has an inherent cross reactivity that will need to be addressed to improve specificity. Another possible explanation is that fluctuations of background intensities in the sector with the *F. tularensis* antibodies were sufficient for the SAR III software algorithm to give false-positive calls.

The BoNT A assay had almost an order of magnitude reduction of sensitivity in multiplex testing compared to the LOD seen in singleplex assays. This result was unexpected, as multiplex immunoassays are not subject to reaction kinetics and limiting reagent problems inherent in PCR-based systems where consistently sensitive multiplex detection is challenging.

A few technical issues also limited sensitivity, requiring some initial trouble-shooting before the formal test plan could be executed. First, a common detergent frequently used in sample buffers for immunoassay (Tween-20) was later confirmed by the vendor to be inhibitory to the assay system. This required the use of a proprietary, vendor-supplied buffer. In addition, there was some ambiguity as to the age and shelf life of the vendor-provided cartridges, calling into question the validity of the initial data demonstrating poor sensitivity. This required additional testing of multiple lots of assay cartridges.

MagPix™

by Luminex, Corp

System Specifications

Vendor: Luminex, Corp.

Website: www.luminexcorp.com

System Cost: $24,000.00

Assay Cost: Price on Request

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: Up to 50

Assay Shelf Life: User determined

Sample Size Required: 20–200 µL

Type of Detection: Antibody

Time to Result: 90–105 minutes for No Wash Assay

System Weight: 38.5 lbs

Operating Range: 50–104 °F (10–40 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). Assay integration requires development and optimization.

System Description

The Luminex MAGPIX utilizes labeled magnetic beads in a fluidics system combined with optical detection and computerized analysis to perform plate-based multiplex immunoassays. Preparation of the sample(s) is done in a 96-well plate on the benchtop or in a biological safety cabinet and consists of multiple incubations with antibody- and fluorescently-labeled magnetic beads. The operator sets assay parameters in xPONENT software on a computer that controls the MAGPIX and loads the assay plate to the MAGPIX, which runs the analysis of the prepared samples. The MAGPIX can run highly multiplexed assays to measure up to 50 different analytes simultaneously in a sample. The MAGPIX also has capability to perform nucleic acid detection utilizing hybridizations to nucleic acid-labeled magnetic beads and has developed a prototype sample preparation cartridge that would automate sample preparation. For this testing, all assays were developed by the laboratory scientists at ECBC.

Test Bed Review

MAGPIX had one of the largest footprints of the biological identifiers, including computer, magnetic bead separator and a plate shaker. Despite the utilization of a faster "no-wash" ELISA-type assay format, the assays still took approximately 2 hours. The software required training and practice to become comfortable at running assays and analyzing the data. MAGPIX assays utilized the same antibodies as other systems in this assessment, yet were more sensitive than most other devices. Limits of detection were approximately 10^5 CFU/ mL sample for bacteria assays, 10^7 PFU/mL sample for virus assays and 1 ug/mL for toxin assays.

Because of its size, the MAGPIX was assessed in the Mobile Laboratory, but not the field setting. Mobile lab end-users gave mostly split opinions (among FAIR, GOOD and EXCELLENT scores) on the MAGPIX. The exception was for "Ease of Use" which rated FAIR, since this instrument requires some training and more sample preparation than most other biological agent identifiers. One operator with flow cytometry experience was comfortable with the MAGPIX and affirmed, "The MAGPIX is similar to other Luminex devices." The perceived safety was ranked either FAIR or GOOD due to operators concerns over handling the 96-well plates with possible pathogens. Users felt they could minimize the safety concerns by extensive use of bleach and the biological safety cabinet within the Mobile laboratory. The operators suggested, "The MAGPIX should be used as a confirmatory technology, after an initial positive PCR assay."

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	N/A	○	$1.00x10^5$
Yersinia pestis	N/A	○	$1.00x10^5$
Vaccinia	N/A	○	$1.00x10^7$
Venezuelan Equine Encephalitis	N/A	○	$1.00x10^8$
Clostridium Botulinum Toxin	N/A	○	$1.00x10^3$
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$1.00x10^5$	✗	$1.00x10^7$
Yersinia pestis	$1.00x10^5$	△	$1.00x10^6$
Vaccinia	$1.00x10^7$	✗	$>1.00x10^8$
Venezuelan Equine Encephalitis	$1.00x10^8$	✓	$1.00x10^8$
Clostridium Botulinum Toxin	$1.00x10^3$	✓	$1.00x10^3$

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

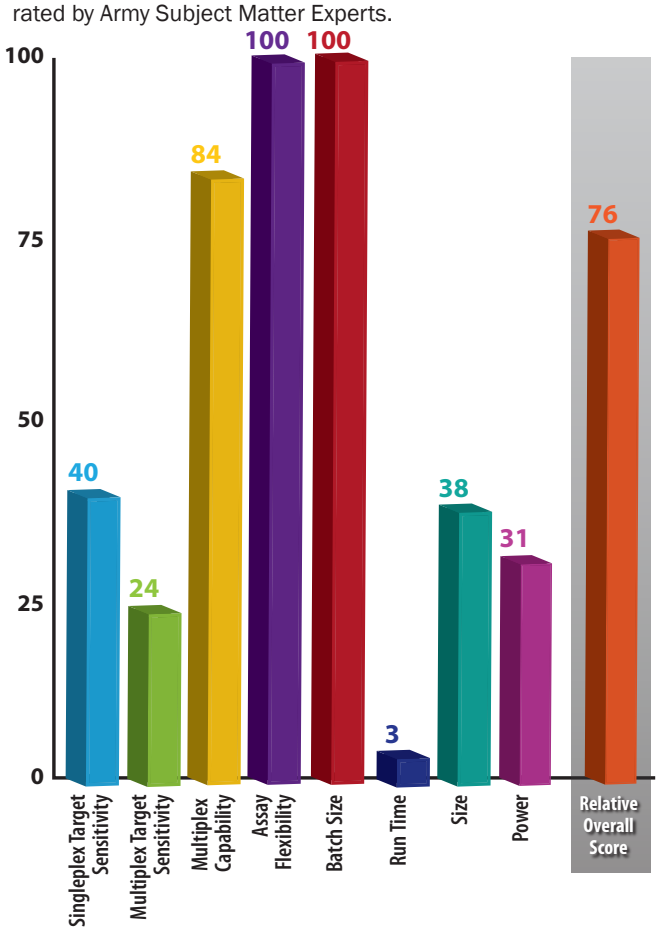
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

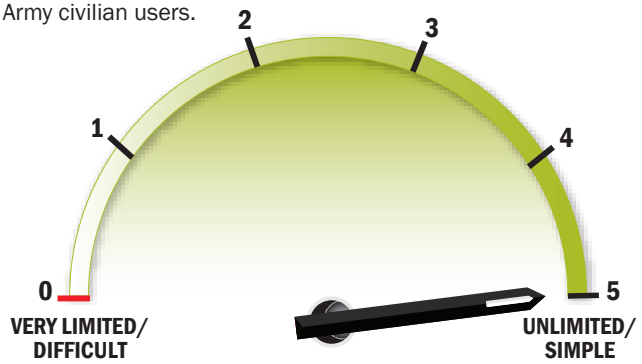
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Overview

The Luminex MAGPIX utilizes labeled magnetic beads in a fluidics system combined with optical detection and computerized analysis to perform plate-based multiplex immunoassays. Preparation of the sample(s) is done in a 96-well plate on the benchtop or in a biological safety cabinet and consists of multiple incubations with antibody- and fluorescently-labeled magnetic beads. The operator sets assay parameters in xPONENT software on a computer that controls the MAGPIX and loads the assay plate to the MAGPIX, which runs the analysis of the prepared samples. The MAGPIX can run highly multiplexed assays to measure up to 50 different analytes simultaneously in a sample. The MAGPIX is also capable of performing nucleic acid detection utilizing hybridizations to nucleic acid-labeled magnetic beads and Luminex has developed a prototype sample preparation cartridge that would automate sample preparation. For this testing, all assays were developed by the laboratory scientists at ECBC.

The Luminex MAGPIX platform running multiplex xMAP® magnetic bead-based assays with existing CRP antibody pairs was tested to evaluate the technology for the detection of five biothreat agents (two bacterial, two viral, and one protein toxin). Custom assays were designed by coupling a capture, monoclonal antibody to a MagPlex bead via a carbodiimide linkage and labeling detection antibodies with biotin via an N-hydroxysulfosuccinimide bond containing a 22Å spacer. Assays were optimized to determine the best concentrations of both the labeled detector antibodies and streptavidin-R-phycoerythrin (SAPE) conjugate. Individual, singleplex assays were performed to determine the LOD for each assay using a no-wash format. Briefly, the antibody coupled beads were blocked with 1% bovine serum albumin (BSA) in PBS then mixed with dilutions of target. Detection antibodies were added followed by the SAPE conjugate with 30 minute incubations in the dark at ambient temperature with 800 revolutions per minute (rpm) shaking between each step. The volume of each assay component was 25µL; therefore, the final volume of the assay was 100µL. Just prior to analyzing the assay on the MAGPIX, the beads were trapped with a magnet and washed twice with PBS + 0.1% Tween-20 and resuspended in 100µL wash buffer. Seventy-five µL of each reaction was analyzed and the average mean fluorescent intensity (MFI) was recorded. Time to result is approximately two hours with this assay. Each concentration was tested in triplicate and each individual sample was tested in triplicate for a total of nine data points per concentration. The MFI of the individual replicates were averaged and the average MFI was compared to the average MFI of a no-antigen sample (background). Samples were considered positive if the MFI was greater than three times the average background MFI.

The xMAP magnetic bead technology has completely open assay architecture with flexible surface covalent conjugation chemistry, allowing for user-developed immunoassay (including serology), PCR, and enzymatic assay configurations.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Gamma irradiated *B. anthracis* spores were diluted to $1.00x10^4$, $1.00x10^5$, and $1.00x10^6$ CFU/mL into block solution (1% BSA in PBS) and tested in triplicate with the custom *B. anthracis* assay. Background of the *B. anthracis* assay was higher than the other four custom assays but was only approximately 20 MFI. The detector antibody was added at 8µg/mL while the SAPE conjugate was added at 16µg/mL. Limit of detection of the assay was found to be $1.00x10^5$ CFU/mL and average MFIs ranged 116 to 155. The MFI of all nine replicates at this concentration was greater than 60.

Table 35. *Bacillus anthracis* LOD

Concentration (Colony Forming Units (CFU)/mL)	Total CFU	Total Genome Equivalents (GE)	Results (Positives/Total Runs)
$1.00x10^4$	$2.50x10^2$	$5.95x10^2$	0/9
$1.00x10^5$	**$2.50x10^3$**	**$5.95x10^3$**	**9/9**
$1.00x10^6$	$2.50x10^4$	$5.95x10^4$	9/9

Vendor Claimed LOD: N/A

Yersinia pestis

Gram negative, rod-shaped bacterium

Gamma irradiated *Y. pestis cells* were diluted to $1.00x10^4$, $1.00x10^5$, and $1.00x10^6$ CFU/mL into block solution (1% BSA in PBS) and tested in triplicate with the custom *Y. pestis* assay. Background of the *Y. pestis* assay was low, approximately 13 MFI. The detector antibody was added at 8µg/mL while the SAPE conjugate was added at 16µg/mL. Limit of detection of the assay was found to be $1.00x10^5$ CFU/mL. The average MFIs ranged from 77 to 99. The MFI of all nine replicates at this concentration was greater than 39.

Table 36. *Yersinia pestis* LOD

Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
$1.00x10^4$	$2.50x10^2$	$3.46x10^2$	0/9
$1.00x10^5$	**$2.50x10^3$**	**$3.46x10^3$**	**9/9**
$1.00x10^6$	$2.50x10^4$	$3.46x10^4$	9/9

Vendor Claimed LOD: N/A

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Gamma irradiated VAC viral particles were diluted to $1.00x10^6$, $1.00x10^7$, and $1.00x10^8$ PFU/mL into block solution (1% BSA in PBS) and tested in triplicate with the custom VAC assay. Background of the VAC assay was low, approximately 13 MFI. The detector antibody was added at 4µg/mL while the SAPE conjugate was added at 16µg/mL. Limit of detection of the assay was found to be $1.00x10^7$. The average MFIs ranged 75 to 111. The MFI of all nine replicates at this concentration was greater than 39.

Table 37. Vaccinia LOD

Concentration (Plaque Forming Units (PFU)/mL)	Total PFU	Total GE	Results (Positives/Total Runs)
$1.00x10^6$	$2.50x10^4$	$3.61x10^4$	0/9
$1.00x10^7$	**$2.50x10^5$**	**$3.61x10^5$**	**9/9**
$1.00x10^8$	$2.50x10^6$	$3.61x10^6$	9/9

Vendor Claimed LOD: N/A

Venezuelan Equine Encephalitis

+ sense ssRNA, Alphavirus

Gamma irradiated VEE viral particles were diluted to $1.00x10^5$, $1.00x10^6$, $1.00x10^7$, $1.00x10^8$, and $1.00x10^9$ PFU/mL into block solution (1% BSA in PBS) and tested in triplicate with the custom VEE assay.

Background was low, approximately 12 MFI. The detector antibody was added at 2µg/mL while the SAPE conjugate was added at 16µg/mL. Limit of detection of the assay was found to be $1.00x10^8$ PFU/mL—average MFIs ranged 3241 to 9717. The MFI of all nine replicates at this concentration was greater than 36. There was a steep decline in intensity for this assay as the antigen was diluted and the assay has much more variance in the individual intensities than the other assays. At $1.00x10^7$ PFU/mL, for example, there is a 100-fold difference in the intensities of some of the individual replicates. Further development and optimization of this assay is needed to enhance the performance of this assay but could not be completed within the scope of this study.

Table 38. Venezuelan Equine Encephalitis LOD

Concentration (PFU/mL)	Total PFU	Total GE	Results (Positives/Total Runs)
$1.00x10^5$	$2.50x10^3$	$2.50x10^3$	1/9
$1.00x10^6$	$2.50x10^4$	$2.50x10^4$	4/9
$1.00x10^7$	$2.50x10^5$	$2.50x10^5$	4/9
$1.00x10^8$	**$2.50x10^6$**	**$2.50x10^6$**	**9/9**
$1.00x10^9$	$2.50x10^7$	$2.50x10^7$	9/9

Vendor Claimed LOD: N/A

Clostridium botulinum Type A toxin

Protein toxin

Active, BoNT A complex was diluted to $1.00x10^0$, $1.00x10^1$, $1.00x10^2$, and $1.00x10^3$ng/mL into PBS and tested in triplicate with the custom BoNT A assay. Background for the assay was the lowest of all five assays developed – approximately 8 MFI. The detector antibody was added at 4µg/mL while the SAPE conjugate was added at 8µg/mL. Note, the SAPE conjugate was added at half concentration in this assay relative to the other four assays which likely led to the lowest background. The LOD of the assay was found to be 1,000ng/mL. The average MFIs ranged from 248 to 274. The MFI of all nine replicates at this concentration was greater than 24.

Table 39. *Clostridium botulinum* toxin Limit of Detection LOD

Concentration (nanograms (ng)/mL)	Total Toxin (ng)	Results (Positives/Total Runs)
$1.00x10^0$	0.025	0/9
$1.00x10^1$	0.25	0/9
$1.00x10^2$	2.5	0/9
$1.00x10^3$	**25**	**9/9**

Vendor Claimed LOD: N/A

Multiplex Detection

The multiplex assessment of the five targets using the MAGPIX demonstrated critical interferences between the assays. For the multiplex evaluation, no additional optimization was performed and assays were tested with the same conditions as had been developed for the singleplex detection. The exception was the BoNT A assay, which for the singleplex required 8µg/mL SAPE conjugate while all other assays required 16µg/mL. The higher amount of conjugate was used for the assessment. The multiplex assays were not fully optimized by ECBC staff (as is normally done for MAGPIX multiplex assays) as this was outside the scope of the testing. The results of this study may have been more favorable if multiplex assay optimization had been performed.

A preliminary multiplex assessment was performed without VEE and BoNT A. For that assay, triplicate samples were created with a mixture of $1.00x10^5$ CFU/mL *B. anthracis*, $1.00x10^5$ CFU/mL *Y. pestis*, and $1.00x10^7$ PFU/mL VAC. Each sample was tested in triplicate for a total of nine individual replicates and the replicates for each sample were averaged. *Y. pestis* was detected in all three samples while *B. anthracis* and VAC were not.

Multiplex assessment was next performed to detect all five targets. Triplicate samples were created with a mixture of $1.00x10^6$ CFU/mL *B. anthracis*, $1.00x10^5$ CFU/mL *Y. pestis*, $1.00x10^8$ PFU/mL VAC, $1.00x10^8$ PFU/mL VEE, and $1.00x10^3$ng/mL BoNT A. Each sample was tested in triplicate for a total of nine replicates and the intensity values for each sample were averaged. *B. anthracis* was not detected in any sample or replicate. *Y. pestis* and VAC were each detected in two of the three samples and seven of the nine replicates. VEE was detected in all three samples, but only eight of the nine replicates, while BoNT A was detected in all samples and replicates.

The concentrations of *B. anthracis* and VEE were increased 10-fold while the concentrations of the other three antigens were kept the same. The multiplex assay was repeated with the altered sample. VAC could not be tested at a higher amount due to the concentration of the stock vial. Detector antibody mix or SAPE conjugate was accidently not added to one well, which caused that well to be negative for all agents. The results of this well were not used to determine sample averages nor included in the individual replicate results. *B. anthracis*, *Y. pestis*, VEE, and BoNT A were detected in all three samples; VAC was not detected in any. With the exception of VAC, for which no replicates were positive, all eight replicates were positive for all agents.

Table 40. Multiplex Evaluation

Agent	Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
B. anthracis	$1.00x10^5$	$2.50x10^3$	$5.95x10^3$	0/9
	$1.00x10^6$	$2.50x10^4$	$5.95x10^4$	0/9
	$1.00x10^7$	$2.50x10^5$	$5.95x10^5$	7/8
Y. pestis	$1.00x10^5$	$2.50x10^3$	$3.48x10^3$	16/18
	$1.00x10^6$	**$2.50x10^4$**	**$3.48x10^4$**	**8/8**
Agent	**Concentration (PFU/mL)**	**Total PFU**	**Total GE**	**Results (Positives/Total Runs)**
VAC	$1.00x10^7$	$2.50x10^5$	$3.60x10^5$	0/9
	$1.00x10^8$	$2.50x10^6$	$3.60x10^6$	7/17
VEE	$1.00x10^8$	$2.50x10^6$	N/D	8/9
	$1.00x10^9$	**$2.50x10^7$**	**N/D**	**8/8**
Agent	**Concentration (ng/mL)**	**Total Toxin (ng)**		**Results (Positives/Total Runs)**
BoNT A	**$1.00x10^3$**	**$2.50x10^1$**		**17/17**

Discussion

Call Assignments

Individual beads are bar-coded with two dyes (red and near infra-red) with over 500 permutations, 50 of which can be used on the MAGPIX platform. Signal is generated via LED excitation of phycoerythrin labeled antibody/DNA detection probe. Beads are imaged via CCD, capturing three-color signatures simultaneously to de-multiplex and quantify signal.

The user sets the threshold for positive call assignments, usually as a function of the background plus a multiple of noise or standard deviation of unbound beads. Analysis is performed either on the on-board software package or exported to be analyzed externally. Because of the statistical analysis used to assign positive and negative calls, each assay is usually performed in triplicate. The data above is presented in aggregate, with three assays performed in triplicate for a total of nine data points.

Assay Development

Step 1: *Selection of antibody pairs*

The antibody pairs for MAGPIX assay development were obtained from inventory offered by the CRP. The particular antibody pair for each target had previously been verified by the CRP to effectively detect the target in sandwich-type immunoassays. The individual antibodies that were selected, with CRP catalogue identifier and production number, are described in the introduction.

Step 2: *Coupling of capture antibodies to MagPlex beads*

The monoclonal capture antibodies were coupled to MagPlex microspheres by a carbodiimide reaction of the primary antibody amino groups to the carboxyl functional groups on the microsphere surface using an xMAP Antibody Coupling Kit at 5µg antibody per $1x10^6$ microspheres. Microspheres were resuspended to 5,000 beads per microliter and stored at 4C protected from light. Coupling was assessed by diluting each microsphere to 50 beads per microliter and blocking the beads overnight in a PBS + 1% BSA solution. The labeled, blocked microspheres were then reacted with titrated anti-Mouse-Phycoerythrin (PE) and analyzed on the MAGPIX instrument. MFI versus concentration of anti-Mouse-PE was plotted and the curve determined to visually match the manufacturer's expected shape.

Step 3*: Biotinylation of detector antibodies*

The detector antibodies were biotinylated using a Thermo Fisher EZ Link Sulfo-LC-NHS Biotinylation Kit at 290µg/mL then buffer exchanged into PBS using 2mL Zeba Spin Desalting Columns. The level of biotinylation was measured using a Thermo Fisher Biotin Quantitation Kit. Incorporation of biotin (mM Biotin per mM Antibody) was measured to be anti-*B. anthracis*: 1.86; anti-*Y. pestis*: 7.33; anti-VAC: 8.64; anti-VEE: 8.55; and anti-BoNT A: 5.42. Labeled antibodies were stored at 4°C.

Step 4: *Checkerboard Assay Development for Optimization of Detector and SAPE concentration*

A checkerboard assessment was performed for each assay to determine optimum concentrations of the biotinylated detector antibody and SAPE conjugate. The antibody coupled microspheres were diluted to 100 beads/µL and blocked overnight with PBS+ 1% BSA. 25µL of blocked beads were pipetted into a 96-well plate with round bottomed wells and mixed with antigen diluted into block (or PBS for BoNT A) at a concentration expected to be detected. The plate was incubated on a plate shaker in the dark at ambient temperature at 800 rpm for 30 minutes. Detector antibody was titrated in two-fold increments into block and 25µL was added to each well. The plate was again incubated as previously described. SAPE was titrated in two-fold increments into block and 25µL was added to each well. The plate was again incubated as previously described. The plate was placed on a magnetic bead separator and the beads were allowed to adhere for 30 seconds. With the plate still on the separator, the liquid was decanted and the wells were washed twice with 100µL wash buffer (PBS + 0.1% Tween-20). The plate was removed from the separator and the beads were resuspended in 100µL wash buffer by pipetting up and down. 75µL of each well was analyzed on the MAGPIX instrument with an additional internal wash step with MAGPIX Drive Fluid. Optimal biotinylated antibody and SAPE concentrations were chosen by comparing the median fluorescent intensity for each concentration of detector or SAPE. The optimal concentrations are summarized in the table below.

Table 41. Optimal Concentrations of the biotinylated detector antibody and conjugate

Assay	Biotinylated Detector Antibody (µg/mL)	SAPE conjugate (µg/mL)
B. anthracis	8	16
Y. pestis	8	16
VAC	4	16
VEE	2	16
BoNT A	4	8

Step 5: *Comparison of washed to no-wash assay*
The recommended protocol for assays developed for the MAGPIX includes a microsphere trapping and wash step following each incubation. The impact of removing the intermediate wash steps was determined to be minimal using the *Y. pestis* assay as a benchmark. Washing the plate did result in slightly increased median intensities and reduced the "hook effect" observed with high concentrations of antigen. The slight increase in median intensity could result in a lower LOD by increasing the intensity of marginally negative samples above the three-times background threshold, but the background of the washed assay was higher than the no-wash assay. It was determined that the increase in assay time and effort to perform the washing steps was not worth the minimal increases in assay performance.

Step 6: *Preliminary LOD*
A preliminary LOD of each assay was determined by titrating the antigen in 10-fold increments and testing each dilution in triplicate with the detector and SAPE at concentrations previously deemed optimal. The preliminary LOD was set as the concentration of antigen for which each of the triplicate samples were positive. A positive response was recorded if the median intensity of the sample was greater than three times the average intensity of triplicate no-antigen samples.

Assay Sensitivity

The Luminex MAGPIX assays were among the most sensitive of all antibody-based systems. The sensitivity for singleplex assays was similar to that for the MSD point of care Cartridge Reader. The LOD for the *B. anthracis* assay was found to be $1.00x10^5$ CFU/mL, while the LOD for *Y. pestis* was found to be $1.00x10^5$ CFU/mL. The sensitivities of these "home-made" MAGPIX bacteria assays is similar to the high end of enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassays (LFIA), with LODs for bacteria in the 10^4-10^5 CFU range. The viral assay LODs were found to be $1.00x10^7$ for VAC and $1.00x10^8$ for VEE which is in the 10^6-10^8 PFU/mL range for viruses seen with ELISA and LFIAs. The MAGPIX BoNT A assay LOD was found to be 1µg/mL, which is at the low end of most ELISA and LFIA sensitivities.

The multiplex assays for bacterial and viral targets were approximately ten-fold less sensitive than for the individual singleplex assays, while the toxin assay did not lose sensitivity. One possible explanation for the BoNT A assay retaining sensitivity in the multiplex assays was the increase in SAPE conjugate to 16µg/mL from 8µg/mL in the singleplex assay. The bacterial and viral multiplex assays did not have a similar increase in the SAPE conjugate compared to the singleplex assays, and thus, experienced lower sensitivity. As stated previously, the multiplex assays were not fully optimized by ECBC staff. The results of this study may have been more favorable if multiplex assay optimization had been performed.

Cartridge Reader

by Meso Scale Discovery (MSD)

System Specifications

Vendor: Meso Scale Discovery (MSD)

Website: www.meso-scale.com

System Cost: Price on Request

Assay Cost: Price on Request

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: 5

Assay Shelf Life: 1 year at 4 °C

Sample Size Required: 165 µL

Type of Detection: Antibody

Time to Result: 30 minutes

System Weight: 13 lbs

Operating Range: 59–86 °F (15–30 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System had successful operation in Mobile lab and field. Prototype/Beta test unit.

System Description

The Cartridge Reader is one of the few non-COTS systems included in this testing. MesoScale Diagnostics' (MSD) Cartridge Reader uses Multi-Array electrochemiluminescence (ECL) technology to provide highly-sensitive multiplexed detection in a small volume of liquid sample. The instrument carries out measurements using single-use injection-molded fluidic cartridges that can conduct multiplexed measurements of up to 12 targets in a sample. Each cartridge has integrated positive and negative controls. Integrated fluidics on-board the cartridge allow for fully automated sample processing and analysis.

Test Bed Review

As a top performer and end user favorite, the Cartridge Reader was easy to use, featured multiplex capability and consistently performed throughout the entire testing.

Achieving detection levels down to $1x10^5$ cfu/ml in both its *Bacillus anthracis* and *Yersinia pestis* singleplex assays, the Cartridge Readers' sensitivity was comparable to other fully developed antibody-based system. In addition to performing well in their singleplex assay design, MSD also scored well in our "Multiplex" category. This technology was able to detect all five targets with no loss of signal when combined in a duplex format. During the initial laboratory testing, the user interface displayed raw data and required exporting the data to an EXCEL macro file to calculate the final results.

Prior to field testing, MSD was contacted and asked to modify the user interface so that results were easily interpretable by the end user. MSD was able to perform a firmware upgrade and testing within one week. In the field, the end-users were impressed with the ease of set-up and minimum amount of training required. Soldiers referred to the system as "Superior" and a "Favorite" of the antibody based systems. One end-user commented that the system seemed "almost too easy". Where this system lacks the sensitivity of a PCR-based technology it excels in its ability to analyze up to 12 different agents in one sample.

Admittedly the current configuration of this system was not intended for outdoor use because it offers no battery option and the system has not been ruggedized. However these characteristics were minor concerns for the end-users who viewed this system as one of their top picks.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	$1.00x10^{5}$	✓	$1.00x10^{5}$
Yersinia pestis	$1.00x10^{7}$	✓	$1.00x10^{5}$
Vaccinia	$1.00x10^{5}$	✗	$1.00x10^{7}$
Venezuelan Equine Encephalitis	$1.00x10^{8}$	✓	$1.00x10^{8}$
Clostridium Botulinum Toxin	$7.00x10^{-2}$	✗	$1.00x10^{2}$
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	$1.00x10^{5}$	✓	$1.00x10^{5}$
Yersinia pestis	$1.00x10^{5}$	✓	$1.00x10^{5}$
Vaccinia	$1.00x10^{7}$	✗	$>1.00x10^{8}$
Venezuelan Equine Encephalitis	$1.00x10^{8}$	✓	$1.00x10^{8}$
Clostridium Botulinum Toxin	$1.00x10^{2}$	△	$1.00x10^{3}$

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

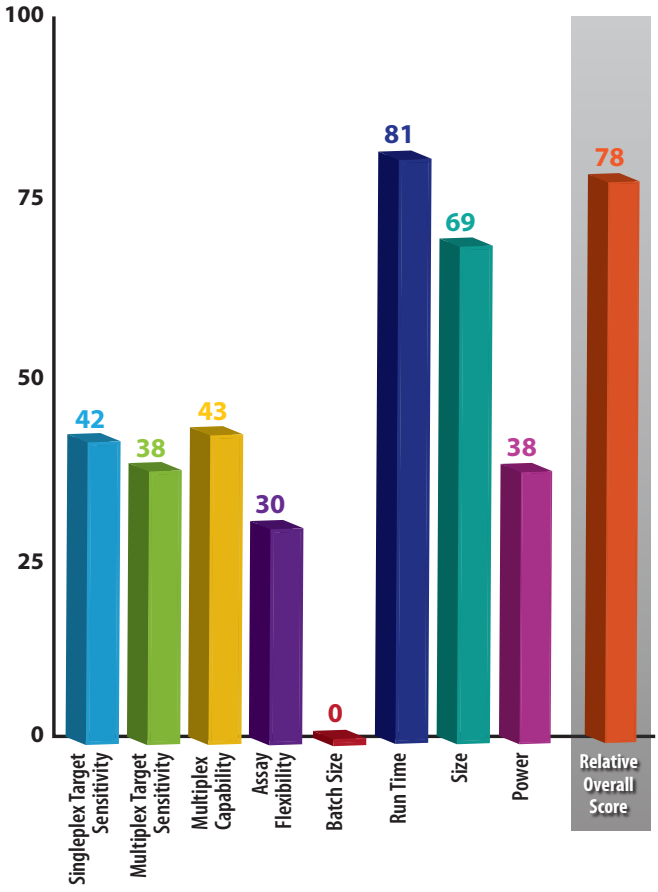
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

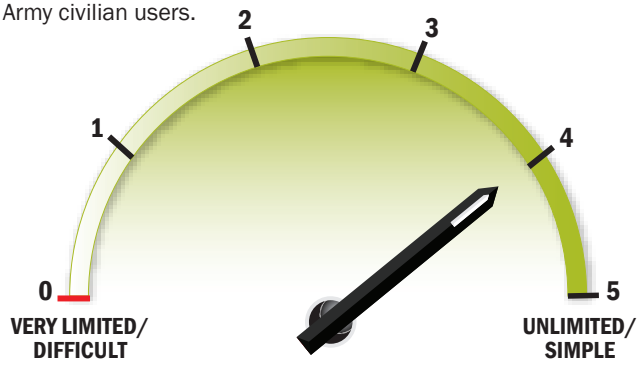
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



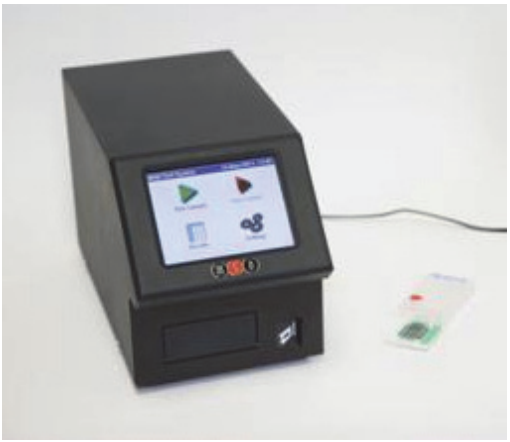
Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Meso Scale Discovery

Overview



The Cartridge Reader is one of the few non-COTS systems included in this testing. MSD Cartridge Reader uses Multi-Array® electrochemiluminescence technology to provide highly-sensitive multiplexed detection in a small volume of liquid sample. The instrument carries out measurements using single-use injection-molded fluidic cartridges that can conduct multiplexed measurements of up to 12 targets in a sample. Each cartridge has integrated positive and negative controls. Integrated fluidics on-board the cartridge allow for fully automated sample processing and analysis.

The Cartridge Reader was tested to evaluate the technology and verify vendor claims of LOD for five biothreat agents (two bacterial, two viral, and one protein toxin). The Cartridge Reader is a simple to use device that processes cassettes containing assays for each target using microfluidics. A user pipets 165µL of sample into the sample port, caps the port, and inserts the cassette into the device. Results are available in approximately 30 minutes and are displayed on the device screen as positive or negative. The customized cartridges contained assays for all five targets studied in this evaluation split between two channels within the cassette. Each channel contains a negative and a positive control for quality control. There is space for a total of six assays plus a positive and negative control on each channel; therefore, a cassette could be developed to test for 12 targets simultaneously.

The LODs reported for each target were taken from the results of Joint Biological Tactical Detection System (JBTDS) Technical Readiness Evaluation (TRE) 09-1 and are the lowest concentration that yielded 100% detection of the agent. The LOD of the BoNT A assay is from work funded by the National Institutes of Health (NIH)/National Institute of Allergy and Infectious Disease (NIAID) and is for agent in buffer.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Gamma-irradiated *B. anthracis* was diluted serially in 10-fold increments into water and tested based on the flow chart in Figure 6 beginning with the most concentrated sample and progressing toward the most dilute sample until a negative result was obtained. Because the $1.00x10^4$ CFU/mL sample was negative, $1.00x10^5$ CFU/mL was tested in triplicate. All three samples were detected positive; therefore, the LOD of the device was determined to be $1.00x10^5$ CFU/mL, which is in agreement with the JBTDS TRE data supplied by the vendor.

Table 42. *Bacillus anthracis* LOD

Concentration (Colony Forming Units (CFU)/mL)	Total CFU	Total Genome Equivalents (GE)	Results (Positives/Total Runs)
$1.00x10^4$	$1.65x10^3$	$3.93x10^3$	0/1
$1.00x10^5$	**$1.65x10^4$**	**$3.93x10^4$**	**3/3**
$1.00x10^6$	$1.65x10^5$	$3.93x10^5$	1/1
$1.00x10^7$	$1.65x10^6$	$3.93x10^6$	1/1

Vendor Claimed LOD: $1.00x10^5$ CFU/mL

Yersinia pestis

Gram negative, rod-shaped bacterium

Gamma-irradiated *Y. pestis* was diluted serially in 10-fold increments into water and a single sample at each dilution was screened beginning with $1.30x10^8$ CFU/mL and progressing to $1.30x10^2$ CFU/mL. Because the $1.30x10^4$ CFU/mL sample was the first negative obtained, two additional tests at that concentration and at the three concentrations immediately above it were completed to determine the consistency of the results. The LOD of the device was determined to be $1.30x10^5$ CFU/mL. This is 100-fold better than was determined during the JBTDS TRE study, but it is more in line with both the *B. anthracis* results reported above and the expectations of a bacterial capture immunoassay.

Table 43. *Yersinia pestis* LOD

Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
$1.30x10^2$	$2.15x10^1$	$2.98x10^1$	1/1
$1.30x10^3$	$2.15x10^2$	$2.98x10^2$	0/1
$1.30x10^4$	$2.15x10^3$	$2.98x10^3$	0/3
$1.30x10^5$	**$2.15x10^4$**	**$2.98x10^4$**	**3/3**
$1.30x10^6$	$2.15x10^5$	$2.98x10^5$	3/3
$1.30x10^7$	$2.15x10^6$	$2.98x10^6$	3/3
$1.30x10^8$	$2.15x10^7$	$2.98x10^7$	1/1

Vendor Claimed LOD: $1.00x10^7$ CFU/mL

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Gamma-irradiated VAC viral particles were diluted serially in 10-fold increments into water and tested based on the flow chart in Figure 6 beginning with the most concentrated sample and progressing to the most dilute sample until a negative result was obtained. Because the $1.00x10^{6}$ CFU/mL sample was negative, $1.00x10^{7}$ CFU/mL was tested in triplicate. All three samples were detected positive; therefore, the LOD of the device was determined to be $1.00x10^{7}$ CFU/mL. This LOD is 100-fold higher than the LOD determined during the JBTDS TRE.

Table 44. Vaccinia LOD

Concentration (Plaque Forming Units (PFU)/mL)	Total PFU	Total GE	Results (Positives/Total Runs)
$1.00x10^{6}$	$1.65x10^{5}$	$2.38x10^{5}$	0/1
$1.00x10^{7}$	**$1.65x10^{6}$**	**$2.38x10^{6}$**	**3/3**
$1.00x10^{8}$	$1.65x10^{7}$	$2.38x10^{7}$	1/1

Vendor Claimed LOD: $1.00x10^{5}$ PFU/mL

Venezuelan Equine Encephalitis

+ sense ssRNA, Alphavirus

Gamma-irradiated VEE viral particles were diluted serially in 10-fold increments into water and tested based on the flow chart in Figure 6, beginning with the most concentrated sample and progressing to the most dilute sample until a negative result was obtained. The first test, performed using $1.00x10^{8}$ PFU/mL, was expected to be positive based on results from VAC testing. The first replicate was positive, so testing was continued at $1.00x10^{7}$ PFU/mL. This sample was negative. Two additional tests at $1.00x10^{8}$ PFU/mL were then performed. All three $1.00x10^{8}$ PFU/mL samples were detected positive; therefore, the LOD of the device was determined to be $1.00x10^{8}$ CFU/mL, which is in agreement with the JBTDS TRE data.

Table 45. Venezuelan Equine Encephalitis LOD

Concentration (PFU/mL)	Total PFU	Total GE	Results (Positives/Total Runs)
$1.00x10^{7}$	$1.65x10^{6}$	$1.65x10^{6}$	0/1
$1.00x10^{8}$	**$1.65x10^{7}$**	**$1.65x10^{7}$**	**3/3**

Vendor Claimed LOD $1.00x10^{8}$ PFU/mL

Clostridium botulinum Type A toxin

Protein toxin

Active BoNT A was diluted serially in 10-fold increments into PBS and tested beginning at the reported LOD and progressing as described in Figure 6. After getting negative results for all three dilutions of BoNT A, fresh dilutions were prepared the following day. A 100ng/mL sample had previously been shown to be positive on the system, so instead of testing 70pg/mL, additional 100ng/mL samples were tested. All three $1.00x10^{2}$ng/mL samples were detected positive; therefore, the LOD of the device was determined to be $1.00x10^{2}$ng/mL, nearly 1,500-fold higher than had been reported by MSD. Of note during this assessment, the 70pg/mL sample was reported, falsely, positive for VAC.

Table 46. *Clostridium botulinum* toxin Limit of Detection LOD

Concentration (nanograms (ng)/mL)	Total Toxin (ng)	Results (Positives/Total Run)
$7.00x10^{-1}$	$1.16x10^{-1}$	0/1
$7.00x10^{0}$	$1.16x10^{0}$	0/1
$1.00x10^{2}$	**$1.65x10^{1}$**	**3/3**

Vendor Claimed LOD: 70pg/mL

Multiplex Detection

Samples containing all four inactivated, gamma irradiated agents were made and tested in triplicate on the custom assay designed by MSD. All four agents were detected in all three samples at the same concentrations that had been detected in the singleplex assessment. This assessment was repeated with samples containing BoNT A as described below.

Samples containing all agents diluted to the concentration found to be the LOD during singleplex testing were made and tested in triplicate on the custom assay designed by MSD. The sample contained $1.00x10^{5}$ CFU/mL *B. anthracis*, $1.00x10^{5}$ CFU/mL *Y. pestis*, $1.00x10^{7}$ PFU/mL VAC, $1.00x10^{8}$ PFU/mL VEE, and $1.00x10^{2}$ng/mL BoNT A. *B. anthracis*, *Y. pestis*, and VEE were detected in all three samples, whereas VAC was detected in only one and BoNT A was detected in none. Fresh triplicate samples containing agents at the same concentration as above. except VAC which was at $1.00x10^{8}$ PFU/mL and BoNT A which was at $1.00x10^{3}$ng/mL. were made and again tested on the custom designed MSD assay cartridge. All agents were detected in all three samples with the modified concentrations. For multiplex detection, it appears there is interference between the BoNT A and VAC detection assays.

Table 47. Multiplex Evaluation 1 - Without *C. botulinum* Type A toxin

Agent	Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
B. anthracis	$1.00x10^{5}$	$1.65x10^{4}$	$3.93x10^{4}$	3/3
Y. pestis	$1.00x10^{5}$	$1.65x10^{4}$	$2.29x10^{4}$	3/3
Agent	**Concentration (PFU/mL)**	**Total PFU**	**Total GE**	**Results (Positives/Total Runs)**
VAC	$1.00x10^{7}$	$1.65x10^{6}$	$2.38x10^{6}$	3/3
VEE	$1.00x10^{8}$	$1.65x10^{7}$	$1.65x10^{7}$	3/3

Table 48. Multiplex Evaluation 2 - With *C. botulinum* Type A toxin

Agent	Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
B. anthracis	**$1.00x10^{5}$**	**$1.65x10^{4}$**	**$3.93x10^{4}$**	**6/6**
Y. pestis	**$1.00x10^{5}$**	**$1.65x10^{4}$**	**$2.29x10^{4}$**	**6/6**
Agent	**Concentration (PFU/mL)**	**Total PFU**	**Total GE**	**Results (Positives/Total Runs)**
VAC	$1.00x10^{7}$	$1.65x10^{6}$	$2.38x10^{6}$	1/3
	$1.00x10^{8}$	**$1.65x10^{7}$**	**$2.38x10^{7}$**	**3/3**
VEE	**$1.00x10^{8}$**	**$1.65x10^{7}$**	**$1.65x10^{7}$**	**3/3**
Agent	**Concentration (ng/mL)**	**Total Toxin (ng)**		**Results (Positives/Total Runs)**
BoNT A	$1.00x10^{2}$	N/D	N/D	0/3
	$1.00x10^{3}$	**N/D**	**N/D**	**3/3**

Meso Scale Discovery

Discussion

Call Assignments

Individual call assignments are made by algorithm analysis of light emitted from the electrochemiluminescence signal. The assay format in the cartridge is two parallel chambers with eight-plex arrays of individual sandwich immunoassays, electrical stimulation of a coordinated Ru^{2+} detection reagent, and capture of emitted light at 620 nm by CCD camera. The arrays are configurable, as demonstrated by the ability of the manufacturer to rapidly develop custom content from CRP's supply of specific antibody reagents.

Assay Sensitivity

The electrochemiluminescence assay technology employed with the Cartridge Reader offers two advantages over traditional fluorescence-based sandwich immunoassay. The excitation of signal is electrical rather than light, allowing for multiple excitations per reporter molecule without the worry of photobleaching and reduced signal over time. There is also no signal loss or ambiguity due to spectral overlaps and related color-deconvoluting signal processing. Both of these characteristics would be expected to enhance signal to noise ratios, resulting in more sensitive and reliable detection.

However, the sandwich immunoassay format is still subject to limitations inherent in antibody-based technologies such as non-specific binding, shared antigen reactivity in near-neighbor species, and cross-reactivity in conserved non-linear epitope motifs. All of these issues can be minimized by blocking, affinity purification (positive and negative selection), and targeted monoclonal antibody production. Each of these modifications has trade-offs in terms of sensitivity, specificity, cost, and development time, which will affect system performance.

The sensitivity of the system is in line with traditional ELISA and LFIA performance, with LODs for bacteria in the 10^4-10^5 CFU range, 10^6-10^7 PFU for viruses, and >1ug/mL for protein toxins. There was a false-positive result for VAC in the singleplex BoNT A testing, which is similar to a noted specificity issue seen during the JBTDS TRE 09-1. An unexpected loss in sensitivity at higher levels of multiplexing was noted for both VAC and BoNT A. In theory, multiplex immunoassays should not have the sensitivity loss often seen with multiplex PCR systems caused by the difficulty in balancing multiple simultaneous enzymatic processes. Possible sources of this loss of sensitivity could be competition for reporter reagents, design issues with the cartridge resulting in lower signal to noise ratios, or interference seen during this testing between BoNT A- and VAC-specific antigen-antibody pairings.

RAPTOR™ Plus

by Research International, Inc.

System Specifications

Vendor: Research International, Inc.

Website: www.researchintl.com

System Cost: $49,500.00

Assay Cost: $150

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: 4

Assay Shelf Life: 1 year at 4 °C

Sample Size Required: 1-2 mL

Type of Detection: Antibody

Time to Result: 28 minutes

System Weight: 14.6 lbs (with battery)

Operating Range: 34 - 95 °F (1 - 35 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System completed but had recurring technical issues.

System Description

The RAPTOR by Research International is a field-ready ruggedized fluorometric assay system about the size and weight of a car battery that can be used to detect biological agents, chemical contaminants or explosives. For biological agent identification, the RAPTOR uses a four channel wave-guide system with specific capture antibodies bound to an immunoassay "coupon" for detection and identification of potential threat agents. The operator must prepare and emplace the detector antibody tubes, a pouch with running buffer and a waste pouch prior to operation. The RAPTOR has two internal peristaltic pumps that control the fluids' movements and the assay progression. The total time for set-up, system test and establishment of a pre-operational baseline reading is approximately 30 minutes, while the sample run time is 14 minutes. The instrument is not quantitative; however, detection signals are displayed as *negative, suspect, positive*, and *highly positive* results, providing some indication of relative quantity of a particular target.

Test Bed Review

The laboratory assessment for the RAPTOR utilized its Bioassay Coupon kit, which included assays for *Bacillus anthracis*, *Yersinia pestis*, Vaccinia virus and BoNT A. The technical staff at Research International was not able to develop a working assay for Venezuelan Equine Encephalitis virus. The RAPTOR did not fare well in the laboratory assessment as positive results were not obtained at all for *Bacillus anthracis* or Vaccinia virus at up to 100x the claimed LOD, while *Yersinia pestis* was only positive at 5 x 10^7 CFU/mL, or 10x the claimed LOD. The RAPTOR required some practice to become adept at making the proper fluidics connections prior to operation. The tubing was prone to kink and color coded connectors were mismatched. Also, an interior module that holds the detector antibody tubes had to be frozen prior to operation, possibly causing inconsistent assay conditions as the instrument warmed throughout the day.

The RAPTOR was assessed in the Mobile Laboratory and by both field operator groups. However, 5 of the 8 operators were unable to complete sample analysis because the RAPTOR failed the fluidics pre-operational testing and would not operate correctly, or failed during the sample analysis. Consequently, the operators' rating of "Ease of Use" varied. The manipulation of tubing and pouches prior to sample analysis was difficult for operators in multiple glove layers. One operator reflected, "You shouldn't have to be a mechanic to set-up the device." The RAPTOR received consistently positive ratings for data viewing and interpretation because the surface display is simple to read and understand.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX

Agent	Vendor Claimed LOD	Validation of Claimed LOD	Actual LOD
Bacillus anthracis	$5.00x10^4$-$5.00X10^5$	✗	$>1.00x10^7$
Yersinia pestis	No Claim	○	$5.00x10^7$
Vaccinia	$1.00x10^5$	✗	$>1.00x10^7$
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	1-10	✗	$1.00x10^4$

MULTIPLEX

Agent	Actual Singleplex LOD	Actual LOD Achieved	Multiplex LOD
Bacillus antracis	$>1.00x10^7$	✗	$>5.00x10^6$
Yersinia pestis	$5.00x10^7$	✓	$5.00x10^7$
Vaccinia	$>1.00x10^7$	✗	$>1.00x10^7$
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

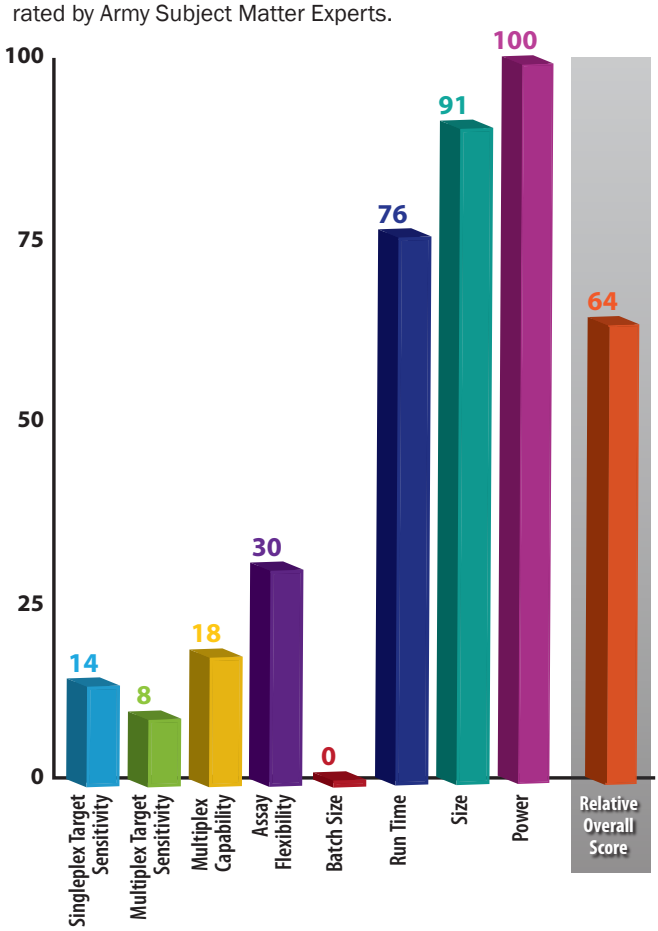
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

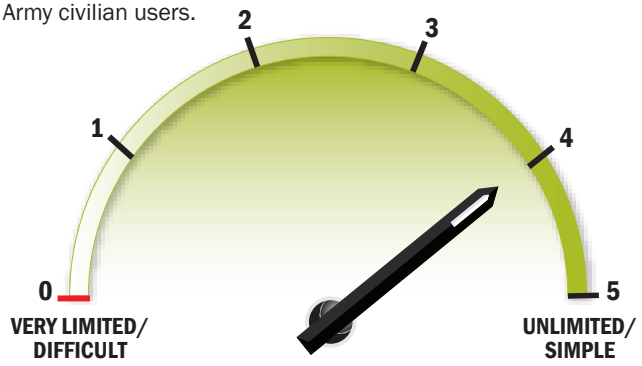
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Overview

The RAPTOR by Research International is a field-ready ruggedized fluorometric assay system about the size and weight of a car battery that can be used to detect biological agents, chemical contaminants, or explosives. For biological agent identification, the RAPTOR uses a four channel wave-guide system with specific capture antibodies bound to an immunoassay "coupon" for detection and identification of potential threat agents. The operator must prepare and emplace the detector antibody tubes, a pouch with running buffer and a waste pouch prior to operation. The RAPTOR has two internal peristaltic pumps that control the fluids' movements and the assay progression. The total time for set-up, system test, and establishment of a pre-operational baseline reading is approximately 30 minutes, while the sample run time is 14 minutes. The instrument is not quantitative; however, detection signals are displayed as *negative*, *suspect*, *positive*, and *highly positive* results, providing some indication of relative quantity of a particular target.

The Research International RAPTOR four channel wave-guide immunoassay system running the Bioassay Coupon kit, which includes assays for *Bacillus anthracis*, *Yersinia pestis,* VAC virus, and BoNT A, was tested to evaluate the technology and verify vendor claims of LOD for four biothreat agents (two bacterial, one toxin, and one viral). The RAPTOR was designed to allow users to set-up the device, move it into a field setting, and operate continuously for up to 24 hours on battery power, but can also be used in a laboratory setting with an A/C adaptor. A single Bioassay coupon kit can process up to 30 samples if all results are negative. A new kit must be installed after each suspicious or positive result. The coupon contains four wave-guides, each functionized with a capture antibody. The device has a fluidics compartment into which the user loads rehydrated fluorophore-labeled detector antibodies, a bag containing running buffer, and a waste bag. Fluidics are manipulated within the device by the use of two peristaltic motors. Complete set-up, performance of a system test that checks for proper function of optical and fluidic components, and obtaining a baseline, takes approximately 30 minutes. A sample is processed in 14 minutes, assuming the baseline is already established during instrument set-up. The system requires large inoculums of sample of 1-2mL which are added directly to a stainless steel sample inlet.

Decontamination of the RAPTOR after a positive detection of an agent may prove problematic. The stainless steel sample port could easily be damaged by bleach solutions. Additionally, the reagents are recycled during sample analysis so that another sample can quickly be analyzed if the initial sample is negative. Therefore, if an agent is present but below the LOD, all four detector antibodies would be contaminated. Finally, the instructions indicate that in the event of a positive test, the assay coupon should be removed and replaced by a "cleaning coupon." Without first decontaminating the fluidic components of the device with the analysis coupon in place, the cleaning coupon would also become contaminated with agent.

Another drawback of the device was the lack of any internal controls or standards that are run with the samples. Without an internal control, there was no way to distinguish a true negative result from a false negative caused by the presence of an inhibitory sample contaminant or to distinguish a true positive result from a false positive result caused by the presence of a contaminant with intrinsic fluorescent properties.

The individual detector antibodies included in the kit are in tubes capped with colored lids, but the colors of the kit components do not match the colors of the reagent tubing assemblies. This led to confusion when setting up the instrument. Additionally, two field users had issues with kinked tubing when the fluidic compartment lid was put in place. All users of the instrument had issues with bubbles detected by the system at random times—most often while completing a system test--which led to the device aborting system test, baseline, and sample runs.

Unfortunately, the system performance, lack of internal controls, and utility issues, such as tubing kinks and color mismatches, render this instrument all but unusable for the intended purpose.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Gamma-irradiated *B. anthracis* spores were serially diluted into water (as indicated by manufacturer data) in 10-fold increments and tested using the RAPTOR Bioassay Coupon kit. The testing summarized in the table below represents several days of testing because of the number of errors generated by the device during testing. During an initial assessment of the device, samples at $1.00x10^6$ and $1.00x10^7$ CFU/mL were evaluated. The $1.00x10^6$ CFU/mL sample was negative while the $1.00x10^7$ CFU/mL sample was high positive (HI+). The assessment of the vendor claim of LOD was then performed. The first $5.00x10^4$ sample was assayed but the device failed because of bubbles just prior to the end of the run. The power for the device was cycled and the system reset. The second $5.00x10^4$ CFU/mL sample was then applied to the same coupon. The positive result obtained may be due to the initial sample remaining on the chip and combining with the agent in the second sample. Testing was resumed after a delay trying to determine the cause of the bubble errors and began with a $5.00x10^4$ CFU/mL and progressed to higher concentrations as described in Figure 6. There was not enough stock antigen available to test $5.00x10^7$ CFU/mL in triplicate—it was expected to be positive based on earlier results with $1.00x10^6$ CFU/mL. Subsequent testing with samples diluted to $1.00x10^6$ and $1.00x10^7$ CFU/mL was performed, each in duplicate because they had been tested previously. *B. anthracis* was not detected in any of the four samples. The LOD of the *B. anthracis* assay was determined to be greater than $1.00x10^7$ CFU/mL which is much higher, 20-200-fold, that the vendor claimed LOD.

Table 49. *Bacillus anthracis* LOD

Concentration (Colony Forming Units (CFU)/mL)	Total CFU	Total Genome Equivalents (GE)	Results (Positives/Total Runs)
$5.00x10^4$	$5.00x10^4$	$1.19x10^5$	1/3 (Pos)
$5.00x10^5$	$5.00x10^5$	$1.19x10^6$	0/1
$1.00x10^6$	$1.00x10^6$	$2.38x10^6$	0/3
$5.00x10^6$	$5.00x10^6$	$1.19x10^7$	0/1
$1.00x10^7$	$1.00x10^7$	$2.38x10^7$	1/3 (HI+)

Vendor Claimed LOD: $5.00x10^4$ - $5.00x10^5$ CFU/mL

Yersinia pestis

Gram negative, rod-shaped bacterium

Gamma-irradiated *Y. pestis* cells were serially diluted into water (as indicated by manufacturer data) in 10-fold increments and tested using the RAPTOR Bioassay Coupon kit following the testing flow chart, Figure 6. Assessment of the LOD was performed starting at $5.00x10^6$ CFU/mL because of the apparent low sensitivity, as demonstrated by the false negative results of *B. anthracis* testing. While the $5.00x10^6$ CFU/mL sample was negative, the $5.00x10^7$ CFU/mL sample was reported as HI+ for all three tests. It appears the device has a small dynamic range because the results are reported, in increasing concentration of agent, Negative, Suspicious, Positive, and HI+. The LOD of the assay was reported in ng/mL F1 antigen which cannot be extrapolated to CFU/mL. It is unknown whether the level of detection determined in this assessment is equivalent to the LOD reported by the manufacturer.

Table 50. *Yersinia pestis* LOD

Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
$5.00x10^6$	$5.00x10^6$	$6.93x10^6$	0/1
$5.00x10^7$	**$5.00x10^7$**	**$6.93x10^7$**	**3/3 (HI+)**

Vendor Claimed LOD: 1-5ng/mL F1 antigen

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Gamma-irradiated VAC viral particles were serially diluted into assay buffer (8.3mM Phosphate buffer, pH 7.2, 0.05% (w/v) Triton X-100) in 10-fold increments and tested using the RAPTOR Bioassay Coupon kit as described in Figure 6 starting at the vendor claimed LOD. VAC was not detected in any of the samples tested. An additional $1.00x10^7$ PFU/mL sample was analyzed on the device using the High Sensitivity Assay. Again, VAC was not detected. Testing was not completed at concentration higher than $1.00x10^7$ PFU/mL because of the large sample volume required and moderate concentration of VAC stock antigen available. The vendor claimed LOD could not be confirmed.

Table 51. Vaccinia LOD

Concentration (Plaque Forming Units (PFU)/mL)	Total PFU	Total GE	Results (Positives/Total Runs)
$1.00x10^5$	$1.00x10^5$	$1.44x10^5$	0/1
$1.00x10^6$	$1.00x10^6$	$1.44x10^6$	0/1
$1.00x10^7$	$1.00x10^7$	$1.44x10^7$	0/1

Vendor Claimed LOD: $1.00x10^5$ PFU/mL

Venezuelan Equine Encephalitis

+ sense ssRNA, Alphavirus

No Assay Available

Clostridium botulinum Type A toxin

Protein toxin

Active BoNT A was diluted serially in 10-fold increments into PBS and tested beginning at 10-100 times the published LOD because the device had sub-optimal sensitivity when detecting bacterial and viral antigens previously. Testing progressed as described in Figure 6. BoNT A was detected in the 10,000ng/mL sample, but not in the more dilute samples. The $1.00x10^4$ng/mL sample was not tested in triplicate because of the large sample volume required and difficulties decontaminating the instrument due to its microfluidic design. The LOD of the device was determined to be no lower than $1.00x10^4$ng/mL, nearly 1,000 to 10,000-fold higher than the vendor claimed LOD.

Table 52. *Clostridium botulinum* toxin Limit of Detection LOD

Concentration (nanograms (ng)/mL)	Total Toxin (ng)	Results (Positives/Total Runs)
$1.00x10^2$	$1.00x10^2$	0/1
$1.00x10^3$	$1.00x10^3$	0/1
$1.00x10^4$	$1.00x10^4$	1/1

Vendor Claimed LOD: 1-10ng/mL

Multiplex Detection

Multiplex testing was completed with samples containing gamma-irradiated *B. anthracis* and *Y. pestis* because of the large amount of BoNT A needed to include the target in the assessment. VAC was also excluded because it was not detected in any sample assessed. Although *B. anthracis* was not expected to be detected at the concentration used for the multiplex assessment, *B. anthracis* was included to determine whether the presence of additional targets interfered with the detection of *Y. pestis*. The assays were only performed in duplicate because of difficulties with bubbles within the microfluidic lines of the device. It appears that the presence of nonspecific agent does not interfere with the detection of *Y. pestis*, although VAC was falsely identified as being present in one of the samples.

Table 53. Multiplex Evaluation

Agent	Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
B. anthracis	$5.00x10^6$	$5.00x10^6$	$1.19x10^7$	0/2
Y. pestis	$5.00x10^7$	$5.00x10^7$	$6.93x10^7$	2/2 (HI+)

Discussion

Call Assignments

Individual call assignments for organisms on the multiplex tickets are made based upon fluorescent signal derived from sandwich immunoassay with a fluorescent reporter on optical wave-guides. Processing of the signal is performed to derive negative, suspect, positive, and highly positive results. The lack of internal controls made it difficult to evaluate negative results generated at 10 to 100-fold above stated LODs. The observed high rate of false-negative results is concerning, as they give the impression of the absence of agent when it may present at extremely high concentrations. A false positive result in the absence of target is also of concern, as it raises the possibility that high concentration of non-specific protein targets may result in spurious positive results requiring a response. The distinction between system calls may be unreliable with limited dynamic range, as all true positive results were highly positive in single ten-fold dilution above negative results. To troubleshoot the problems with bubbles in the tubing, data was sent to Research International for analysis. Additionally, the device was connected to a computer with diagnostics software, but no root cause was found.

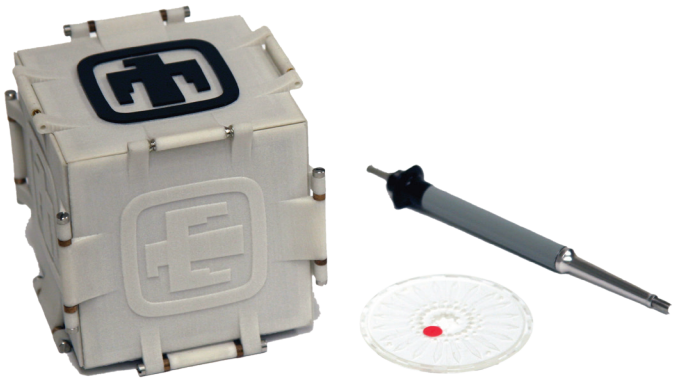
Assay Sensitivity

Assay sensitivity was extremely poor, limited to levels high enough that bulk contamination of the target of interest would be required and the resulting viscosity of the bulk reference materials could limit the very upper limit of testing. Two organisms (*B. anthracis* and VAC) were not detected at any concentration during the evaluation.

The root cause of the lack of sensitivity is unknown, and investigations will be significantly inhibited by the lack of internal controls to discriminate true negatives from assay, instrument, reagent, or consumable failures. One possible source of failure is the delicacy of wave-guide systems, which have been shown to be subject to fouling in non-pristine samples. Fluidics issues and ambiguity of system architecture were also found on the instrument.

SpinDx™

by Sandia National Laboratory



System Description

The SpinDx by Sandia National Laboratory is a non-COTS system designed primarily for rapid diagnostics in a clinical or point-of-care setting. The system utilizes a spinning disk, or "lab on a CD", to draw a sample through a separation matrix while simultaneously binding to fluorescently labeled antibody-bead constructs. The SpinDx uses LED illumination and a photodiode to detect the target. Preliminary results from Sandia show the system to have greater detection sensitivity than standard ELISAs. The SpinDx has also been used to separate whole blood samples for cell counts and other clinical analyses. Operation of the system requires little or no training, and samples require no preparation except for mixing with the analytical matrix. The SpinDx is controlled by a laptop computer via wireless (Bluetooth) communications. With an analysis time of less than 20 minutes, no sample preparation, battery power and small size, the SpinDx has potential to be a prototypical mobile laboratory or even hand-held instrument.

System Specifications

Vendor: Sandia National Laboratory

Website: N/A

System Cost: To be determined (non-COTS device)

Assay Cost: $2 per disk

Assay Storage Requirements: 4 °C

Agents Tested per Assay: Up to 20 possible

Assay Shelf Life: 6 months at 4 °C

Sample Size Required: 2 µL

Type of Detection: Fluorescent labeled antibodies

Time to Result: 20 minutes

System Weight: 3.5 lbs (system also requires laptop)

Operating Range: 59 - 86 °F (15 - 30 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). System is early prototype unit, components are not final. Assay development in progress.

Test Bed Review

The results of the laboratory assessment indicate the SpinDx is still at the developmental prototype stage. Sandia utilized Critical Reagent Program antibodies to create assays for all 5 targets; however, the assays were not able to definitively detect any of the targets in the current configuration of the SpinDx. The analytical matrix was rather gel-like, and adding 3 uL of the sample-matrix mix to the port on the disk was difficult and not precise. Sandia supplied a specific pipet and plastic tips to load the device, but the sample loading step could still be improved. The operation of the device was guided by software on a laptop computer connected wirelessly to the SpinDx. Because of concerns about the software measurement of analytical beads after the "spin", Sandia provided calibration beads. However, the analytical software indicated either the calibration beads did not run properly in the disks or the detection algorithm was errant. The battery re-charger connection interferes with closing of the device lid. The system's sample matrix loading and the analytical software both seemed in need of improvement for environmental sample analysis.

The SpinDx was assessed by the Mobile laboratory operators, but not by the field operators. Because of some difficulty in mixing the sample with analytical matrix and pipeting into the disk port, as well as running the control software, the operators rated the "Ease of Use" and "Data" viewing and interpreting attributes as being only FAIR. The operators rated "Training Simplicity", "Safety" and "Maintenance" all as EXCELLENT. The operators also were favorable to the overall design of the instrument and the short musical tune that denotes completion of an assay.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program.

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	N/A	○	N/A
Yersinia pestis	N/A	○	N/A
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus antracis	N/A	○	N/A
Yersinia pestis	N/A	○	N/A
Vaccinia	N/A	○	N/A
Venezuelan Equine Encephalitis	N/A	○	N/A
Clostridium Botulinum Toxin	N/A	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

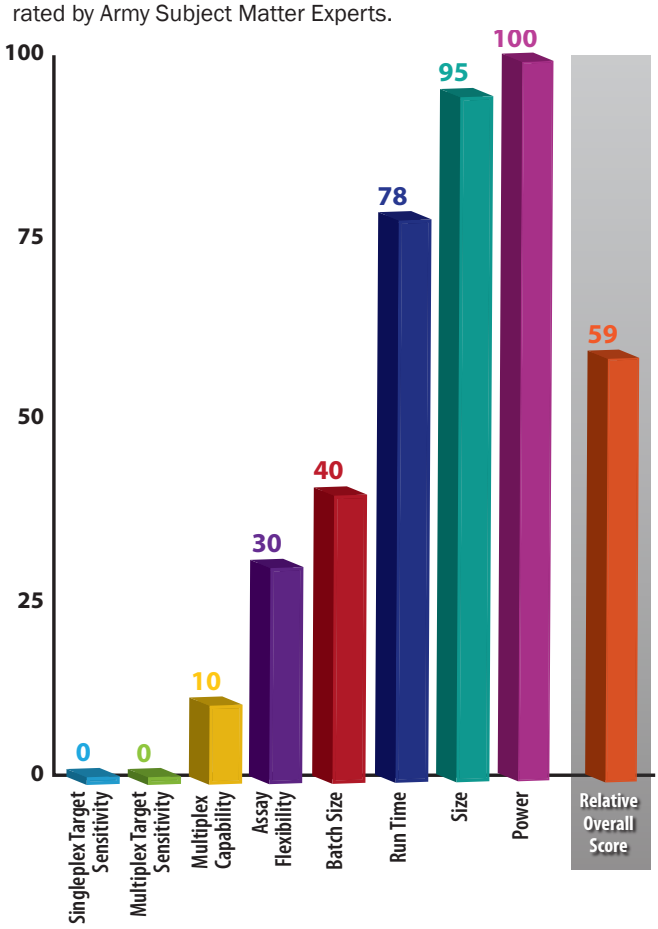
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

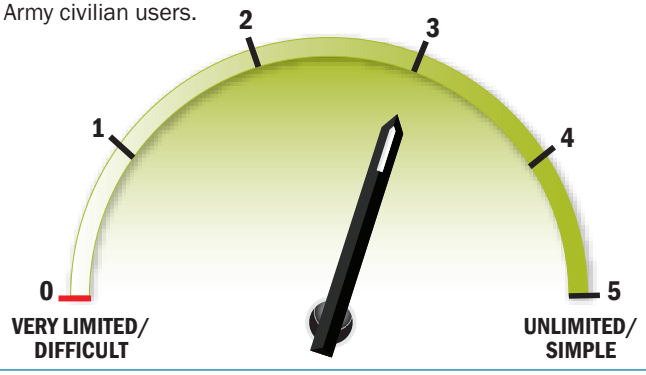
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Overview

The SpinDx by Sandia National Laboratories is a non-COTS system designed primarily for rapid diagnostics in a clinical or point-of-care setting. The system utilizes a spinning disk, or "lab on a CD," to draw a sample through a separation matrix while simultaneously binding to fluorescently labeled antibody-bead constructs. The SpinDx uses LED illumination and a photodiode to detect the target. Preliminary results from Sandia show the system to have greater detection sensitivity than standard ELISAs. The SpinDx has also been used to separate whole blood samples for cell counts and other clinical analyses. Operation of the system requires little or no training and samples require no preparation except for mixing with the analytical matrix. The SpinDx is controlled by a laptop computer via wireless (Bluetooth) communications. With an analysis time of less than 20 minutes, no sample preparation, battery power, and small size, the SpinDx has potential to be a prototypical mobile laboratory or even hand-held instrument.

The SpinDX assay format is a single antibody capture assay contained within a spinning flat disc containing 20 reaction lanes laser etched and radiating from the center of the disc. A sample is applied to a slot near the center of the disc and, as the disc spins, the sample mass passes through a sieving matrix and binds to specific antibodies for a target of interest. The sample-antibody mixture passes through the sieving matrix to the outward point of the sample lane, where fluorescence is read by the photodiode. Each lane, in theory, could support up to 20 assays concurrently based on fluorescence labeling of the antibodies and the capabilities of the photodiode.

The SpinDX provided by Sandia National Laboratories was a prototype device and still under development. While all other instruments in this study had been obtained by the fall of 2012, the SpinDX production was delayed and the prototype device was not delivered until early February 2013, just prior to the mobile laboratory assessment. As such, the SpinDX did not conclusively detect targets among all the samples that were tested. Future iterations of the SpinDX should correct the assay performance problems of the spin disk mechanism.

The SpinDX was assessed in the Mobile laboratory scenario for usability, but not the functionality or ability to accurately detect target agents.

Data

No usable data was obtained. During initial evaluation of *Yersinia pestis* assays, the photodiode signals were not compatible with accurate data collection from the sample disc. Thus, the singleplex and multiplex assays were not performed.

Discussion

Call Assignments

The SpinDX is designed to make detection calls based on an increase in fluorescent signal over baseline levels as detected by the internal photodiode. A laptop computer communicates with the SpinDX via Bluetooth and runs software that controls SpinDX operations and collects and interprets signals from

the device. There is no analysis software included on the present prototype iteration; therefore, detection algorithms are left to the user to develop.

Assay Sensitivity

Since no assays were able to be evaluated, the assay sensitivity is unknown for this prototype version.

SPIRIT™

by Seattle Sensors Corporation

System Specifications

Vendor: Seattle Sensors Corporation

Website: www.seattlesensors.com

System Cost: $35,000.00

Assay Cost: $423.00

Assay Storage Requirements: Refrigeration

Agents Tested per Assay: One

Assay Shelf Life: 6 months at 4 °C

Sample Size Required: 100–150 µL

Type of Detection: Antibody

Time to Result: 5–10 minutes

System Weight: 3 lbs

Operating Range: 59–86 °F (15–30 °C)

Technology Readiness Level (TRL)

The Technology Readiness Level has been determined by a subject matter expert panel analyzing all relevant data and rated according to an interpretation of the Technology Readiness Assessment (TRA) Guidance document prepared by U.S. DoD's ASD (R&E). Software is not yet final. System not tested to full capability.

System Description

The SPIRIT by Seattle Sensors Systems Corporation is a shoe-box sized biological agent identifier that uses surface plasmon resonance (SPR) to detect and measure binding, such as between an antibody and a specific bacteria. The SPIRIT has condensed the research laboratory technique of SPR to a portable device capable of detecting bacteria, viruses or toxins from complex samples by utilizing Texas Instruments' Spreeta SPR chips. A laptop computer is used to control the peristaltic pumps and valves and to regulate the flow of sample and buffer onto the SPR flowcells such that each sample may be analyzed within 25 minutes. The operator monitors the SPR signals through a graphic display and post-run data analysis. The system allows for regeneration of the Spreeta chip surfaces, such that up to 100 samples may be analyzed before the chip must be replaced. Among the mobile and man-portable detection systems, SPIRIT has relatively fast assay times, a small footprint and battery-power.

Test Bed Review

Seattle Sensors Systems developed assays for all 5 test agents through binding antibodies from the Critical Reagent Program to individual SPR chips. The software version included with the SPIRIT did not allow full functionality; consequently, only singleplex detection capability was assessed on this version of the SPIRIT. The laboratory assessment utilized individual targets on channel 4 and a calibration control on channel 3, while channels 1 and 2 were not configured for data collection in this software release. The set-up, priming and calibration of the SPIRIT took about 30 minutes, while sample data collection took approximately 25 minutes. The SPIRIT was at the lower end of sensitivity among the biological agent identifiers as bacteria samples were detected at 10^7 CFU/mL and Vaccinia virus inconsistently identified at 10^7 PFU/mL. Meanwhile, VEE was not detected at 10^8 PFU/mL or BoNT A at 1 ug/mL. The SPIRIT required some training and practice for the operators to become comfortable with performing assays.

The SPIRIT received generally favorable usability scores from Mobile laboratory operators, with an EXCELLENT and FAIR rating for "Ease of Use" and GOOD or EXCELLENT scores for Data, Training, Safety and Maintenance categories. The operators had some troubles with the injection of sample to the port and experienced inconsistent internal peristaltic pump pressures. The SPIRIT was sensitive to work surface vibrations perturbing the data collection. Of importance to sample size requirements, one operator was concerned that "1 mL of sample was needed" which may use too much of their collected sample.

Laboratory Limit Of Detection (LOD) Validation

Singleplex validation of vendor LOD claims and Multiplex replication of singleplex LODs in a laboratory setting. Targets were inactivated pathogens from the U.S. DoD's Critical Reagent Program. (Units: Bacteria = CFU/mL, Virus = PFU/mL, toxin = ng/mL)

SINGLEPLEX			
Agent	**Vendor Claimed LOD**	**Validation of Claimed LOD**	**Actual LOD**
Bacillus anthracis	No Claim	○	>1.00x10^7
Yersinia pestis	No Claim	○	1.00x10^7
Vaccinia	No Claim	○	1.00x10^8
Venezuelan Equine Encephalitis	No Claim	○	>1.00x10^8
Clostridium Botulinum Toxin	No Claim	○	>1.00x10^3
MULTIPLEX			
Agent	**Actual Singleplex LOD**	**Actual LOD Achieved**	**Multiplex LOD**
Bacillus anthracis	>1.00x10^7	○	N/A
Yersinia pestis	1.00x10^7	○	N/A
Vaccinia	1.00x10^8	○	N/A
Venezuelan Equine Encephalitis	>1.00x10^8	○	N/A
Clostridium Botulinum Toxin	>1.00x10^3	○	N/A

✓ Validated △ Not Validated (≤1 log difference) ✗ Not Validated (<1 log difference) ○ No Claim

Man Portable and Mobile Usability

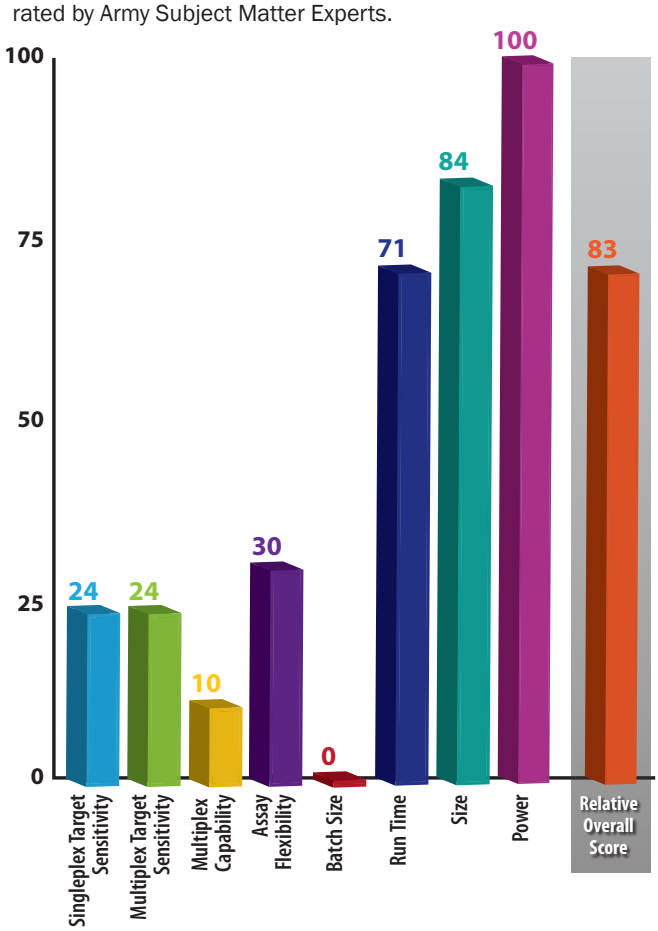
Each technology has been evaluated for usability in the field for hand-held/man portable and mobile laboratory settings. The ratings are based on input from multiple Army field operators and Subject Matter Experts

MAN PORTABLE

MOBILE

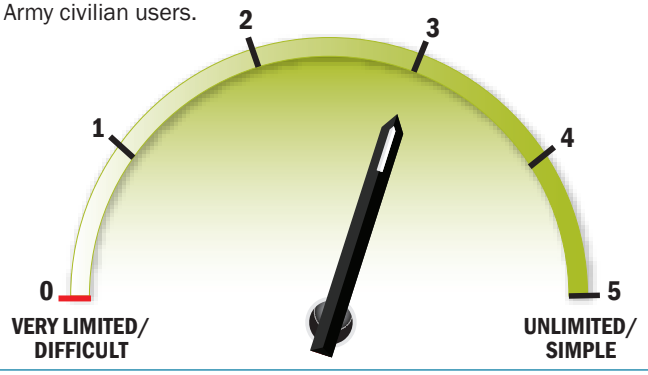
Laboratory Usability Scores

Weighted scoring is based on laboratory data compiled and rated by Army Subject Matter Experts.



Flexibility to Add New Assays

Each technology has been evaluated for assay flexibility as determined by multiple sources of assays and ease of assay development. The rankings are based on input from multiple Army civilian users.



Seattle Sensor Systems
CORPORATION

Overview

The SPIRIT by Seattle Sensors Systems Corporation is a shoe-box sized biological agent identifier that uses surface plasmon resonance (SPR) to detect and measure binding, such as between an antibody and a specific bacteria. The SPIRIT has condensed the research laboratory technique of SPR to a portable device capable of detecting bacteria, viruses or toxins from complex samples by utilizing Texas Instruments' Spreeta SPR chips. A laptop computer is used to control the peristaltic pumps and valves and to regulate the flow of sample and buffer onto the SPR flowcells, such that each sample may be analyzed within 25 minutes. The operator monitors the SPR signals through a graphic display and post-run data analysis. The system allows for regeneration of the Spreeta chip surfaces and up to 100 samples may be analyzed before the chip must be replaced. Among the mobile and man-portable detection systems, SPIRIT has relatively fast assay times, a small footprint, and battery-power.

The Seattle Sensor Systems SPIRIT platform running a customized SPR 2000 (Spreeta) Sensor Chip with existing antibodies was tested to evaluate the technology and verify vendor claims of LOD for five biothreat agents (two bacterial, two viral, and one protein toxin). Four Spreeta chips were installed on the SPIRIT to complete the fluidic circuit and one of the four was assigned as the reference electrode to distinguish signal from nonspecific background. Therefore, the device had the capability of detecting three agents simultaneously plus a single reference. The analysis software, written specifically for this assessment, only monitored Spreeta chips three (reference) and four (test). Because of this limitation, no assessment of multiplex capability was performed. The controller software allowed the user to select one of four pre-programmed methods or control the device manually. During operation, the user was required to install a run buffer, chip reconditioning buffer, and empty waste bottle onto the system, all of which were included in the "kits," and equilibrate the chips and tubing. All of these steps were accomplished using manual controls. Additionally, the antibody coated Spreeta chips were rehydrated in run buffer for two minutes, installed into slot four, and referenced against a high refractive index solution (also included in the kit). Once the chips were equilibrated, the user selected and started the proper program and injected the sample at the appropriate time. The SPIRIT required a minimum 250µL sample to fill an injection loop situated below the instrument panel, but the manufacturer recommended the use of samples of approximately 1mL so that the loop was rinsed and the edge effect caused by the mixing of buffer and sample were minimized. The programs utilized for this assessment were capture assays without signal amplification and were completed in approximately 25 minutes.

In the absence of an expert user, analysis of the run data must be completed utilizing accessory software to determine whether an agent has been detected. The software evaluates the SPR signal during the binding step of the program and outputs a slope, fit, and detection message. The increase in SPR signal during binding is directly proportional to amount of agent present. Slope values greater than 0.010 are considered positive for agent while slope values between 0.005 and 0.0099 are considered indication of potential presence or weak detection. There are no guidelines for acceptable fit values.

The abilities to detect bacterial, viral, and protein toxins and to provide an orthogonal confirmatory technology to more common nucleic acid-based systems are both desirable characteristics of the

system. In addition, SPR is not a commonly used detection scheme, which provided another technology to evaluate for the program.

Unfortunately, the system did not provide consistent and sensitive detection of bacteria, viruses, or protein toxin at any concentration. The system software was difficult to use and poorly documented. Finally, not all of the functionality was tested, as only two of the four instrument channels were available for use because of the analysis software on this SPIRIT version. In effect, the TRL of the instrument could only be considered four to five, well below COTS/GOTS expectations.

Data

Bacillus anthracis

Gram positive, spore forming bacilli

Gamma-irradiated *B. anthracis* was diluted serially in 10-fold increments into Run Buffer (PBS + 0.1% Tween-20) and tested based on the flow chart in Figure 6 beginning at $1.00x10^6$ CFU/mL. *B. anthracis* was not detected in the $1.00x10^6$ CFU/mL sample; therefore, $1.00x10^7$ CFU/mL sample was tested. *B. anthracis* was detected in this sample, so the concentration was tested two additional times. *B. anthracis* was detected in one of the two samples, thus the LOD was stated to be greater than $1.00x10^7$ CFU/mL. Testing of concentrations above this level was not possible due to the large sample volume required.

Table 54. *Bacillus anthracis* LOD

Concentration (Colony Forming Units (CFU)/mL)	Total CFU	Total Genome Equivalents (GE)	Results (Positives/Total Runs)
$1.00x10^6$	$2.50x10^5$	$5.95x10^5$	0/1
$1.00x10^7$	$2.50x10^6$	$5.95x10^6$	2/3

Vendor Claimed LOD: N/A

Yersinia pestis

Gram negative, rod-shaped bacterium

Gamma-irradiated *Y. pestis* was diluted serially in 10-fold increments into run buffer (PBS + 0.1% Tween-20) and tested based on the flow chart in Figure 5Figure 6 beginning at $1.00x10^6$ CFU/mL. *Y. pestis* was not detected in the $1.00x10^6$ CFU/mL sample; therefore, $1.00x10^7$ CFU/mL sample was tested. *Y. pestis* was detected in all three of the triplicate samples, thus the LOD was stated to be $1.00x10^7$ CFU/mL.

Table 55. *Yersinia pestis* LOD

Concentration (CFU/mL)	Total CFU	Total GE	Results (Positives/Total Runs)
$1.00x10^6$	$2.50x10^5$	$3.46x10^5$	0/1
$1.00x10^7$	$2.50x10^6$	$3.46x10^6$	3/3

Vendor Stated LOD: N/A

Vaccinia

dsDNA Orthopox virus, Smallpox [Variola] simulant

Gamma-irradiated VAC was diluted serially in 10-fold increments into run buffer (PBS + 0.1% Tween-20) and tested based on the flow chart in Figure 6 beginning at $1.00x10^7$ PFU/mL. VAC was detected in the first sample, though the fit of the slope was poor, so testing continued with the $1.00x10^6$ PFU/mL sample. The initial sample at $1.00x10^6$ PFU/mL was also positive but the slope appeared much larger than expected (0.072 versus 0.025 for the $1.00x10^7$ PFU/mL sample), there was a large amount of noise in the signal, and the fit was extremely poor (0.278). Therefore, the $1.00x10^6$ PFU/mL sample was repeated rather than testing a $1.00x10^5$ PFU/mL sample. VAC was not detected in the repeat. Additional replicates at $1.00x10^7$ PFU/mL were then tested and agent was detected in neither. After the completion of the final $1.00x10^7$ PFU/mL sample program, the system automatically restarted the program without user input. No additional sample was injected into the sample loop. VAC was detected in this blank sample and the fit was the best of all runs thus far completed. This detection was considered a false positive. A single sample at $1.00x10^8$ PFU/mL was then analyzed. This sample was weakly positive (slope 0.009) so the LOD of the device was considered to be $>1.00x10^8$ PFU/mL. Testing of concentrations above this level was not possible due to the large sample volume required.

Table 56. Vaccinia LOD

Concentration (Plaque Forming Units (PFU)/mL)	Total PFU	Total GE	Results (Positives/Total Runs)
$1.00x10^6$	$2.50x10^5$	$3.61x10^5$	1/2
$1.00x10^{7*}$	$2.50x10^6$	$3.61x10^6$	2/4
$1.00x10^{8**}$	$2.50x10^7$	$3.61x10^7$	1/1

* False positive blank sample observed

** Weak positive signal

Vendor Stated LOD: N/A

Venezuelan Equine Encephalitis

+ sense ssRNA, Alphavirus

Gamma-irradiated VEE viral particles were diluted into run buffer (PBS + 0.1% Tween-20) and tested starting at $1.00x10^8$ PFU/mL because of the poor results obtained with VAC viral particles. VEE was not detected in the $1.00x10^8$ PFU/mL sample, thus the LOD was stated to be greater than $1.00x10^8$ PFU/mL. Testing of concentrations above this level was not possible due to the large sample volume required.

Table 57. Venezuelan Equine Encephalitis LOD

Concentration (PFU/mL)	Total PFU	Total GE	Results (Positives/Total Runs)
$1.00x10^8$	$2.50x10^7$	$2.50x10^7$	0/1

Vendor Stated LOD: N/A

Clostridium botulinum Type A toxin

Protein toxin

Active BoNT A was diluted serially in 10-fold increments into buffer (PBS + 0.1% Tween-20) and tested beginning at 100ng/mL. Testing progressed as described in Figure 6. BoNT A was detected in neither the

100 nor 1,000ng/mL sample. The LOD of the device was determined to be greater than $1.00x10^3$ng/mL. Testing of concentrations above this level was not possible due to the large sample volume required.

Table 58. *Clostridium botulinum* Type A LOD

Concentration (ng/mL)	Total Toxin (ng)	Results (Positives/Total Runs)
$1.00x10^2$	$2.50x10^1$	0/1
$1.00x10^3$	$2.50x10^2$	0/1

Vendor Stated LOD: N/A

Multiplex Detection

Multiplexing was not performed, as the instrument was capable of only analyzing a single reference and test channel at this phase of development.

Discussion

Call Assignments

Systems calls were made from interpretation of SPR signal on a gold surface coated with antibody. Bound antigen in a test channel provides an increase in signal and deflection from unbound antibody signal alone in a reference channel. Calls include "detected," "warning," and "no call," with slope and R^2 values given as fitness metrics. The significance of such metrics is not known. Frequent "bad call" system messages were seen, but referred to as normal by vendor personnel. The instrument's operating system records and displays real-time sensor data, but this data does not provide a system call until the data is analyzed after the run with a separate software package.

Assay Sensitivity

The assay format is SPR detection of a single antibody capture assay. No attempt was made to increase signal, sensitivity, or specificity of the assay using a secondary antibody with an SPR enhancer, such as silver nanoparticles, despite the fact that the vendor does have experience with such systems.

Sensitivity of the system did not detect any of the analytes in a meaningful range, with bacterial CFU LODs of $>10^6$, viral PFU LODs of $>10^8$, and a complete inability to detect BoNT A at any concentration tested. The sensitivity was so poor that additional testing with stock concentrations was considered prohibitively expensive and functionally irrelevant.

Sensitivity of the system could potentially be increased with better utilization of sample, as only one quarter to one half of the total 500-1000µL was contained in the sample fluidics loop for analysis. However, even a two- to four-fold increase in signal by increasing the amount of accessible sample would be insignificant without substantial improvement in assay design.

MOBILE LABORATORY ASSESSMENT

Selection of Systems

At the end of the laboratory assessment subject matter experts (SME) evaluated the instruments for suitability in the field assessment phase. Factors considered to determine the appropriateness for instrument inclusion in the field assessment included, but were not limited to:

- Ease of use
- Ease of result viewing
- East of result interpretation
- Training simplicity
- Safety
- Cleaning

Operators

Two members of the 20th Support Command (SUPCOM), based at Aberdeen Proving Ground, MD performed the mobile laboratory assessment. These operators had extensive experience using analytical equipment to detect biological agents and required minimal training on the equipment prior to testing.

Mobile Laboratory

The 20th SUPCOM employs the HMEL for their mission. The HMEL, which has bench space, power supply, and a temperature controlled environment for multiple instruments to run concurrently, was utilized for this mobile assessment. The staff was supplied with necessary equipment and reagents to perform the assessment.

Sample Construction and Analysis

The mobile laboratory assessment focused on determining the instruments' capacity to be run by typical operators in a mobile laboratory environment. Therefore, sample preparation by the operators was kept to a minimum. Systems that analyze nucleic acids utilized either the inactivated agent, which is processed by integrated sample preparation capability in the instrument prior to analysis or by an affiliated sample dilution or processing apparatus, or purified DNA via a Qiagen DNeasy Kit. Instruments that utilized antibody-based detection technologies received samples containing whole inactivated biological agent in pristine buffer. Samples were prepared at a concentration of ten times the LOD determined in the Laboratory Assessments for each instrument. Each two-man team of operators received a single positive sample per instrument. The operators were able complete assessments for four instruments each day. The operators utilized the Operational Assessment Table (Appendix D) to capture information and opinions on the instruments, in addition to the outcomes of sample analyses.

Results

Operational mobile laboratory assessments culminated in the ranking of each system based on six specific attributes: ease of use; ease of result (viewing data); ease of result (interpretation of data); supporting documentation; training simplicity; safety; and cleaning/maintenance. These rankings were quantified using a weighted scale to provide a final score for field operations based on operator input. Each attribute was ranked by the operator in one of five categories: excellent (five points); good (four points); fair (three points); poor (two points); and lowest (one point). Each of the operators assigned one rank to each attribute.

Using the point system outlined above, a numerical value was assigned that quantifies the user opinion of mobile laboratory operations for each system. The number presented is the percent score the system

received in each attribute. For example, the T-COR 4 received 19 of the possible 20 points for ease of use, thus it received a 95. The overall score from the T-COR 4 is a 94, resulting in an excellent rating.

Table 59. Overall Mobile Laboratory system scores

Technology	System	Ease of Use	Ease of Result Viewing	Ease of Result Interpretation	Training Simplicity	Safety	Cleaning	Overall Score (%)
Nucleic-Acid	BioFire FilmArray	100	100	100	100	100	100	**100**
	BioFire RAZOR EX	90	100	100	100	100	100	**98**
	Epistem Genedrive	70	90	90	100	70	100	**87**
	IQuum Liat	100	100	100	100	100	100	**100**
	Tetracore T-COR 4	90	100	100	90	100	100	**97**
Antibody	ANP NIDS	100	90	90	100	100	100	**97**
	Luminex MAGPIX	60	80	80	90	70	90	**78**
	MSD Cartridge Reader	100	100	100	100	100	100	**100**
	Research Int'l RAPTOR	50	80	80	70	60	70	**68**
	Sandia Nat'l Lab SpinDx	60	60	60	100	100	100	**80**
	Seattle Sensors SPIRIT	80	80	80	90	80	80	**82**

ND – Not Determined. The Mobile laboratory operators did not evaluate all systems supporting documentation

Table 60. BioFire FilmArray

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use					2 of 2
Ease of Result Viewing					4 of 4
Ease of Result Interpretation					4 of 4
Training Simplicity					2 of 2
Safety					2 of 2
Cleaning/Maintenance					1 of 1
Operator Comments: • Sharps are ok. • Biosafety cabinet for setup. • 1 hour is good for mobile. • Less prep than JBAIDS (JBAIDS, 1-2-3 kit extraction) historically. • No toxin. • Prefer 1 sample, full panel. • Reagents expired 12/16/12, system should have caught that.					

Table 61. BioFire RAZOR EX

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use				1 of 2	1 of 2
Ease of Result Viewing					2 of 2
Ease of Result Interpretation					2 of 2
Training Simplicity					2 of 2
Safety					2 of 2
Cleaning/Maintenance					2 of 2
Operator Comments: • Like the color coding. • Chart tells you what's in each well.					

Table 62. Epistem Genedrive

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use			1 of 2	1 of 2	
Ease of Result Viewing				1 of 2	1 of 2
Ease of Result Interpretation				1 of 2	1 of 2
Training Simplicity					2 of 2
Safety			1 of 2	1 of 2	
Cleaning/Maintenance					2 of 2
Operator Comments: • Bubbles were observed in each lane of cartridge. • PCR pellet came out because of static. • Possibility of cross contamination with the card.					

Table 63. IQuum Liat

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use					2 of 2
Ease of Result Viewing					2 of 2
Ease of Result Interpretation					2 of 2
Training Simplicity					2 of 2
Safety					2 of 2
Cleaning/Maintenance					2 of 2
Operator Comments: • Like this one for a presumptive sample. • Not enough variability for an unknown. • Refrigeration is not an issue (they have M1M)					

Table 64. Tetracore T-COR 4

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use				1 of 2	1 of 2
Ease of Result Viewing					2 of 2
Ease of Result Interpretation					2 of 2
Training Simplicity				1 of 2	1 of 2
Safety					2 of 2
Cleaning/Maintenance					2 of 2
Operator Comments: • Cycles ended at different times. • Presumptive ID. • More steps than the Liat, with the same results. • Real time is good, but they would still wait on the negative.					

Table 65. ANP NIDS SAR III

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use					2 of 2
Ease of Result Viewing				1 of 2	1 of 2
Ease of Result Interpretation				1 of 2	1 of 2
Training Simplicity					2 of 2
Safety					2 of 2
Cleaning/Maintenance					2 of 2
Operator Comments: • NIDS is used in presumptive ID by a different group. • Mobile is used more for a definitive ID.					

Table 66. Luminex MAGPIX

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use			2 of 2		
Ease of Result Viewing			2 of 4		2 of 4
Ease of Result Interpretation			2 of 4		2 of 4
Training Simplicity				1 of 2	1 of 2
Safety			1 of 2	1 of 2	
Cleaning/Maintenance				1 of 2	1 of 2
Operator Comments: • Like this one as a screening tool. • Refrigeration is not an issue (they have MIM)					

Table 67. MSD Cartridge Reader

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use					2 of 2
Ease of Result Viewing					2 of 2
Ease of Result Interpretation					2 of 2
Training Simplicity					2 of 2
Safety					2 of 2
Cleaning/Maintenance					2 of 2

Operator Comments:

- Superior system.
- Possible substitute for M1M.
- Low handling.
- Favorite of the antibody systems.
- Easy to use.
- Low possibility of cross contamination.
- 30 min run is good.
- Sound technology.
- Sensitivity is not a driving force.

Table 68. Research International RAPTOR Plus

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use		1 of 2	1 of 2		
Ease of Result Viewing				1 of 1	
Ease of Result Interpretation				1 of 1	
Training Simplicity			1 of 2	1 of 2	
Safety			2 of 2		
Cleaning/Maintenance			1 of 2	1 of 2	

Operator Comments:

- No auto-prompt.
- Does not allow you to insert the coupon incorrectly.
- When cap was removed, there was splashing of a liquid.
- They wouldn't be able to reuse the vials.
- Only tests for 4.
- Waste design – a lot of bags – or clean bags.

Table 69. Sandia National Laboratory SpinDX

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use			1 of 1		
Ease of Result Viewing			1 of 1		
Ease of Result Interpretation			1 of 1		
Training Simplicity					1 of 1
Safety					1 of 1
Cleaning/Maintenance					1 of 1
Operator Comments: • Can't run on power cord at all because the cord prevents the lid from going down & the system turning on. • Centrifuge tube to make a pellet, difficult for pipet tip to pick up 3 µl of prepared sample & reagents. • "Play" button in software not obvious. • Started taking baseline when closed lid and did not wait for software. • Channels 1-5 gave no baseline.					

Table 70. Seattle Sensors SPIRIT

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use			1 of 2		1 of 2
Ease of Result Viewing				2 of 2	
Ease of Result Interpretation				2 of 2	
Training Simplicity				1 of 2	1 of 2
Safety				2 of 2	
Cleaning/Maintenance				2 of 2	
Operator Comments: • Sample volume is unrealistic (1mL).					

FIELD TEST ASSESSMENT

Selection of Systems

During and at the conclusion of the laboratory assessment, operators and SMEs evaluated the instruments for suitability in the field test assessment phase. Factors that determined the appropriateness for instrument inclusion in the field assessment included, but are not limited to:

- Size
- Battery Power
- Sample Preparation Requirements
- Reagent Stability
- End User Needs

Operators and Testing Site

The operators for the man-portable/handheld instrument field test assessments were members of the 56th Chemical Reconnaissance Detachment (CRD) 5th Special Forces Group and the Army 22nd Chemical Battalion. These operators had less extensive experience using analytical equipment to detect biological agents and therefore underwent one day training on the candidate instruments prior to performing this assessment. Testing was performed at Skippers Point Training Area at the Aberdeen Proving Ground, Edgewood Area, MD. This test site is a housing duplex with surrounding yard that is currently used for CBRNE training exercises by the Advanced CBRNE Training Team (See Figures 7 and 8).

Figure 7. Members of the 56th CRD obtain samples at Skippers Point training facility.

Figure 8. Members of the 56th CRD prepare samples.

Sample Construction and Analysis

The field assessment focused on determining the instruments' capacity to be run by typical operators in a non-laboratory environment. Sample preparation by the operators was kept to a minimum. Systems that analyzed nucleic acids utilized either the inactivated agent, which is processed by integrated sample preparation capability in the instrument prior to analysis or by an affiliated sample dilution, processing apparatus, or purified DNA via Qiagen DNeasy Kit. Instruments that utilized antibody-based detection technologies received samples containing whole inactivated biological agent in pristine buffer. Samples were prepared at a concentration of ten times the LOD as determined in the Laboratory Assessments for each instrument. Each two-man team or single operator received a single positive sample per instrument. The operators utilized the Operational Assessment Table to capture information and opinions on the instruments, in addition to the outcomes of sample analyses (see Appendix D).

Results

Operational field assessments culminated in the ranking of each system based on seven specific attributes: ease of use; ease of result viewing; ease of result interpretation of data; supporting documentation; training simplicity; safety; and cleaning/maintenance. These rankings were quantified using a weighted scale to provide a final score for field operations based on operator input. Each attribute was ranked by the operator in one of five categories: excellent (five points); good (four points); fair (three points); poor (two points); and lowest (one point). Each of the operators assigned one rank to each attribute.

Using the point system outlined above, a numerical value that quantifies the user opinion of field operations for each system was assigned. The number presented is the percent score the system received in each attribute. For example, the Liat received 29 of the possible 30 points for ease of use, thus it received a 97. The overall score for the Liat is a 96 resulting in an excellent rating.

Table 71. Overall handheld/man-portable system ratings

System	Ease of Use	Ease of Result Viewing	Ease of Result Interpretation	Supporting Documents	Training Simplicity	Safety	Cleaning	Overall
Liat	97	100	100	90	100	100	80	**96**
Cartridge Reader	100	93	100	100	100	100	100	**99**
Genedrive	73	90	53	60	87	97	90	**79**
NIDS	100	93	93	100	100	83	100	**95**
RAPTOR	60	90	90	90	95	80	75	**82**
RAZOR EX	80	93	93	90	93	93	75	**89**
T-COR 4	97	97	97	100	100	93	90	**96**

Table 72. BioFire Diagnostics RAZOR EX ratings

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use			2 of 6	2 of 6	2 of 6
Ease of Result Viewing				2 of 6	4 of 6
Ease of Result Interpretation				2 of 6	4 of 6
Supporting Documentation				2 of 4	2 of 4
Training Simplicity				2 of 6	4 of 6
Safety				2 of 6	4 of 6
Cleaning/Maintenance			1 of 4	3 of 4	

Operator Comments

- Manipulation of ticket reduced ease of use from Excellent to Good.
- Difficulty using barcode scanner. Suggest a device on barcode scanner for positioning assay at correct distance, orientation, and angle for scanning.
- Comb could be stiffer because tool on end gets damaged before all plungers twisted.
- Assay boxes tough to get into with multiple pair of gloves.
- Device needs to give immediate confirmation that assay has begun running.
- Needs an easy to find progress window.
- Carry strap gets in way when trying to scan barcode.
- Screen hard to read, even with the Brightness button.
- Progress screen should be the default choice.
- Troubleshooting section was in-depth.
- Too many crevices for particles to get into. A Hypewipe could not get into the crevices.
- Sample/buffer ports are too hard to see.
- Audible signal alert that successful scanning of the barcode would be useful.
- Cardboard insert in assay kit exchanged for a foam insert or other device that would hold bottles tighter, allowing one-handed opening of bottles would be an improvement.

Table 73. Epistem Genedrive ratings

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use			2 of 6	4 of 6	
Ease of Result Viewing				3 of 6	3 of 6
Ease of Result Interpretation	2 of 6		2 of 6	2 of 6	
Supporting Documentation			4 of 4		
Training Simplicity			2 of 6		4 of 6
Safety				1 of 6	5 of 6
Cleaning/Maintenance			1 of 4		3 of 4

Operator Comments

- The manual should go over how to interpret and view results.
- Instrument as it is would not be taken "down range"; sample test cartridge would be loaded down range, de-conned, brought back to a cold zone, and ran on an instrument.
- Very impressed with the size.
- Device does not allow input of sample information.
- Operators noted that there were too many fine movements required for use in a tactical situation and that set-up took too long. Device would not be used in field because of limited time on target. Operators noted there were too many accessories.
- Operator 1 had trouble pushing button of Genedrive to start sample run while wearing MOPP gear. Used one of the bead tubes to push button.
  Operator 2 dropped assay cartridges when setting up reactions. In this scenario, operators had unlimited supply of reagents but this may be an issue in the field with limited resources.

Table 74. IQuum Liat ratings

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use				1 of 6	5 of 6
Ease of Result Viewing					6 of 6
Ease of Result Interpretation					6 of 6
Supporting Documentation				2 of 4	2 of 4
Training Simplicity					6 of 6
Safety					6 of 6
Cleaning/Maintenance			1 of 4	2 of 4	1 of 4

Operator Comments

- Troubleshooting was lacking from manual.
- Unit would have to be sent to upper echelon for decontamination. Concerned that particles could get inside the door on top of instrument.
- Would like to see a handle added to the instrument.
- Like the nice contrast for the screen.
- Very easy to use in MOPP gear.
- Barcode reader easy to use, unit very user friendly.
- Operators indicated that if the unit were slightly smaller, it could be a good field use device.
- Prefer a front-loading cartridge.
- Login/Pin is overkill for military application and that stylus and user cards would get lost.
- Liked the accurate count down timer.
- Easy to use.
- Hard keys were difficult to feel through the multiple layers of gloves.
- Screen needs to be tougher.

Table 75. Tetracore T-COR 4 ratings

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use				1 of 6	5 of 6
Ease of Result Viewing				1 of 6	5 of 6
Ease of Result Interpretation				1 of 6	5 of 6
Supporting Documentation					4 of 4
Training Simplicity					6 of 6
Safety			1 of 6		5 of 6
Cleaning/Maintenance			1 of 4		3 of 4
Operator Comments • Backlight on instrument could be brighter. • Centrifuge was still spinning when opened, safety concern. • Instruments looks like it could go down range but would not be eligible because of the inability to be decontaminated. • Real time was excellent. • Results were easily interpreted. • Operator commented that a handle would be preferable.					

Table 76. ANP NIDS SAR III ratings

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use					6 of 6
Ease of Result Viewing				2 of 6	4 of 6
Ease of Result Interpretation				2 of 6	4 of 6
Supporting Documentation					4 of 4
Training Simplicity					6 of 6
Safety		1 of 6	1 of 6		4 of 6
Cleaning/Maintenance					4 of 4
Operator Comments • Monitor easy to read. • Good contrast. • Like that it is small and portable. • Reader makes interpretation of HHA consistent from user to user. Buttons could be a little bigger. • Users are concerned with chance of device becoming contaminated with agent since the cartridge is inserted into the device without any cover over the sample pad. • User noted that device is small enough to fit into pouch on body armor.					

Table 77. MSD Cartridge Reader ratings

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use					6 of 6
Ease of Result Viewing				2 of 6	4 of 6
Ease of Result Interpretation					6 of 6
Supporting Documentation					4 of 4
Training Simplicity					6 of 6
Safety					6 of 6
Cleaning/Maintenance					4 of 4
Operator Comments • Instrument does not have a battery, obviously not for the field. • Run time is very short compared to others. • Ability to test for multiple targets is very good. • Operators concerned whether units build static charge which could spark a chemical or explosive agent in immediate area. • Very little time required to set up equipment.					

Table 78. Research International RAPTOR ratings

Attribute	Test Score				
	Lowest	Poor	Fair	Good	Excellent
Ease of Use	1 of 4		1 of 4	2 of 4	
Ease of Result Viewing				1 of 4	1 of 4
Ease of Result Interpretation				1 of 4	1 of 4
Supporting Documentation				2 of 4	2 of 4
Training Simplicity				1 of 4	3 of 4
Safety			2 of 4		2 of 4
Cleaning/Maintenance			1 of 4	3 of 4	
Operator Comments • Two operators were not able to run a sample due to errors; therefore, no rating for ease of interpretation was given. • Set up was too labor intensive and small parts could get lost. • Tubing was too short and dexterity was a major issue. • Color confusion was an issue. Colors need to match or not be used. • Stainless steel adapters for the reagent vials could potentially puncture their gloves. • Decon would be difficult with this device because sample interacts with interior of device. • Device was too heavy. • Suggestions for improvement include: adding stiffeners to the end of the tubing, color coding the spigots, making colors of reagent vials match the color of the corresponding tubing assembly/cap device.					

SUMMARY OF SYSTEM SCORES

Overall

The relative overall scores represent the cumulative assessment scores that were adjusted so the highest scoring system, the Liat, had a Relative Overall score of 100. The Relative Overall scores of these systems are most notable for aggregating around the 80th percentile. In other words, no particular instrument stood out among the rest as being vastly superior or was a relative failure when considering all aspects of biological agent identification. The IQuum Liat was the highest scoring nucleic acid-based biological identifier, while the ANP NIDS was the highest scoring antibody-based system according to the performance criteria and scoring algorithm.

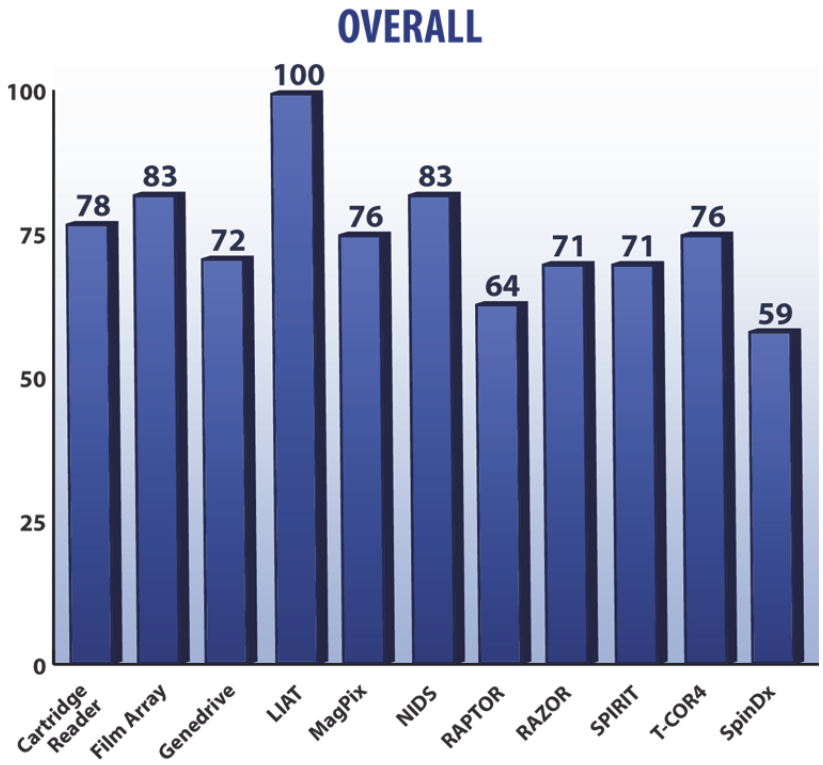


Figure 9. Overall summary of system scores

Singleplex Target Sensitivity

As expected, two nucleic acid-based systems, FilmArray and Liat, scored the best for single target detection sensitivity in spite of being "penalized" for not being capable of detecting the toxin BoNT A. The RAPTOR and SPIRIT were lowest scoring systems, mainly due to their inability to detect all targets consistently at relatively low LODs. Of particular note when comparing sensitivities, the sample size that is actually analyzed varies from system to system. For instance, the T-COR 4 only requires 1 uL of sample per assay tube, while the RAPTOR requires 1-2mL of sample per assay. The Singleplex Sensitivity was determined using concentration of target within the sample, although the actual volume of sample analyzed was different.

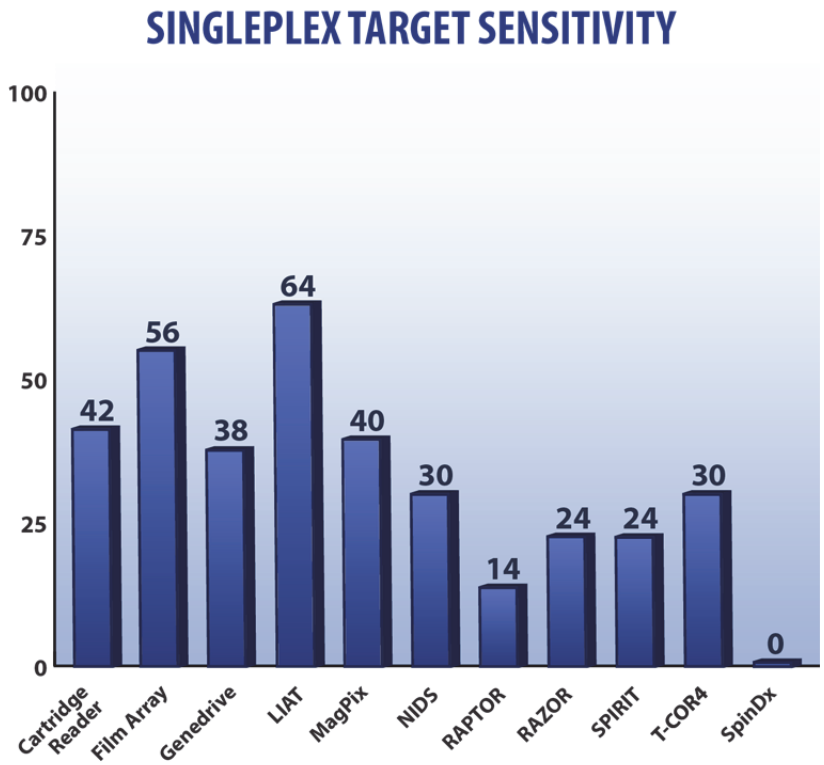


Figure 10. Summary of Singleplex Sensitivity scores

Multiplex Target Sensitivity

In general, the instruments retained nearly the same sensitivity for singleplex targets when multiple targets were in the sample, although instruments without the full complement of multiplex capabilities had lower possible scores in this category. The Liat and FilmArray received relatively high scores for having very high sensitivity for the targets they could identify. Three instruments (Genedrive, SpinDx, and T-COR 4) received scores of zero for multiplex sensitivity because they were unable to simultaneously run assays for multiple targets. Conversely, some instruments could perform duplex (Liat, NIDS, RAZOR EX), triplex (NIDS), or 4-plex (FilmArray), or the full 5-plex analyses (Cartridge Reader, MAGPIX). The RAZOR EX had the capability of detecting 10 targets using the "10-Pouch" assay kit; however, only two of the assessment targets were part of the "10-Pouch" kit.

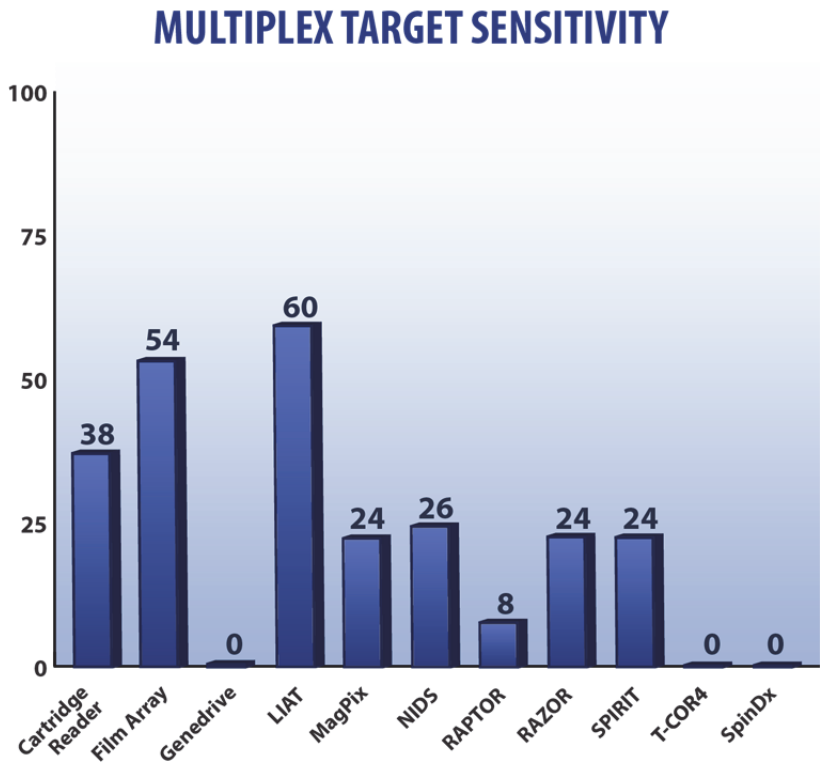


Figure 11. Summary of Multiplex Sensitivity scores

Multiplex Capability

The Multiplex Capability attribute was comprised of the scores for "Number of reportable agents per run" and "Number of individual targets per test" categories. The MAGPIX had the highest score for this attribute because the highly multiplex beads allow up to 50 targets to be simultaneously detected. In contrast, the SPIRIT, as configured, could analyze only one sample at a time for one target. The FilmArray had multiple targets per test for *B. anthracis* (i.e., a chromosomal and two plasmid targets) and other agents, while all other instruments, as tested, were capable of only one target per test agent.

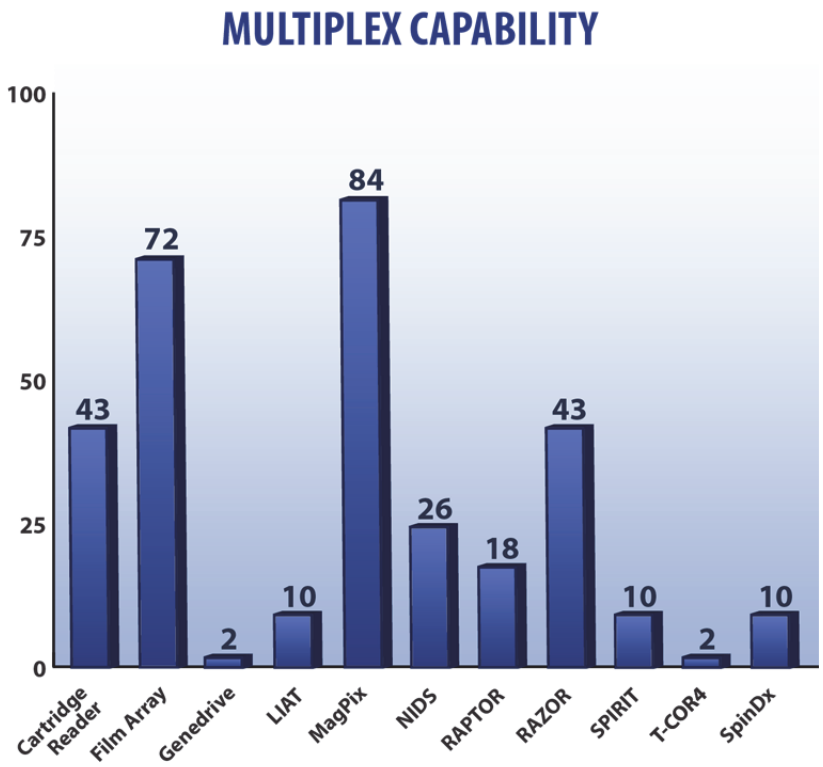


Figure 12. Summary of Multiplex Capability scores

Assay Flexibility

The Assay Flexibility attribute, intended to capture the ease with which new assays could be incorporated to the system, was comprised of the performance criteria scores for "Number of sources of consumables or self-designed assays" and "New assay integration" categories. The MAGPIX and T-COR 4 received maximum scores due to their ability to easily integrate new antibodies or primers, respectively, into new assays. Meanwhile, the FilmArray, RAZOR EX, and Liat were somewhat closed systems in that users would have difficulties and/or delays in adding new assay targets to their menu.

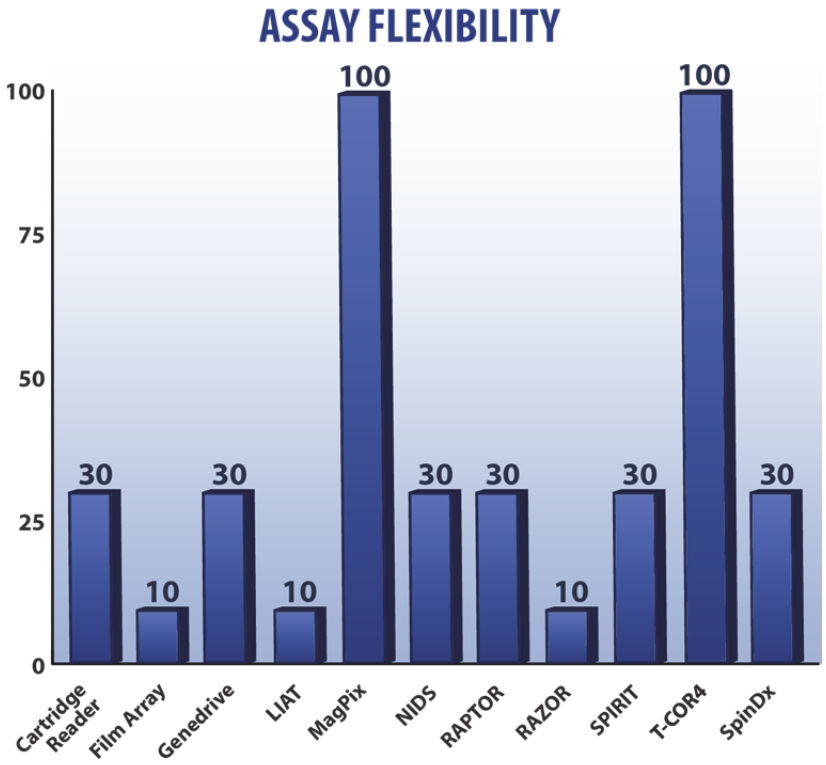


Figure 13. Summary of Assay Flexibility scores

Batch Size

The Batch Size attribute was defined by the following, "If looking for a single agent, the number of samples that can be processed at the same time by one analyzer." The MAGPIX had the highest score for this attribute because the plate format allows for up to 96 samples to be processed at the same time. Most systems, as currently configured, were only able to analyze a single sample at one time.

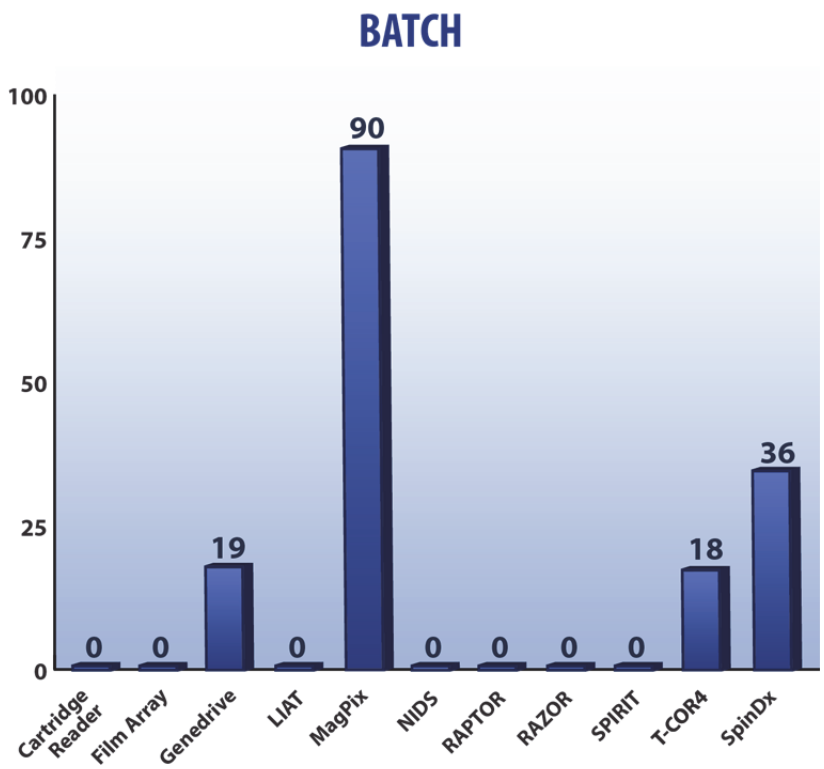


Figure 14. Summary of Batch Size scores

Run Time

The Run Time attribute is a combination of system warm-up time, sample preparation time, and time for analysis of the sample. Several systems scored moderately high as they had combinations of low or no warm-up requirement, no sample preparation, and analysis of less than 35 minutes. The NIDS scored highest as there is no warm-up required, the sample may be directly applied, and the run is complete within 16 minutes. The FilmArray, Genedrive, and T-COR 4 had mediocre scores as PCR requires a relatively longer time to perform. Although both the Liat and RAZOR EX utilize PCR, these systems were able to shave the analysis time for higher scores. The MAGPIX required multiple incubations which pushed the total analysis time over two hours.

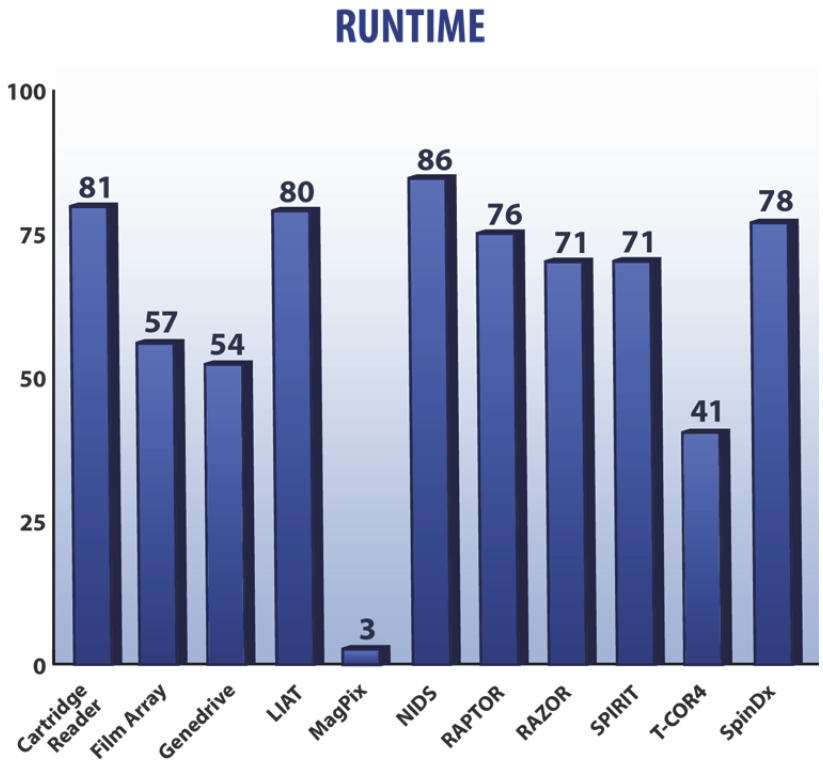


Figure 15. Summary of Run Time attribute scores

Size

The Size score was determined by the system footprint, or area required to operate the system as well as the system weight. Several systems achieved, or nearly achieved, the goals of being less than two pounds and 1.5 square feet. These systems are most appropriate for handheld or man-portable usage. When including other characteristics such as battery power and no ancillary equipment, the Genedrive and NIDS best fit the handheld instrument requirements. The MAGPIX and FilmArray scored lowest in size attributes; however, the MAGPIX is presently a research-oriented instrument and the FilmArray a clinical diagnostic instrument, so they are suitable only for the mobile laboratory.

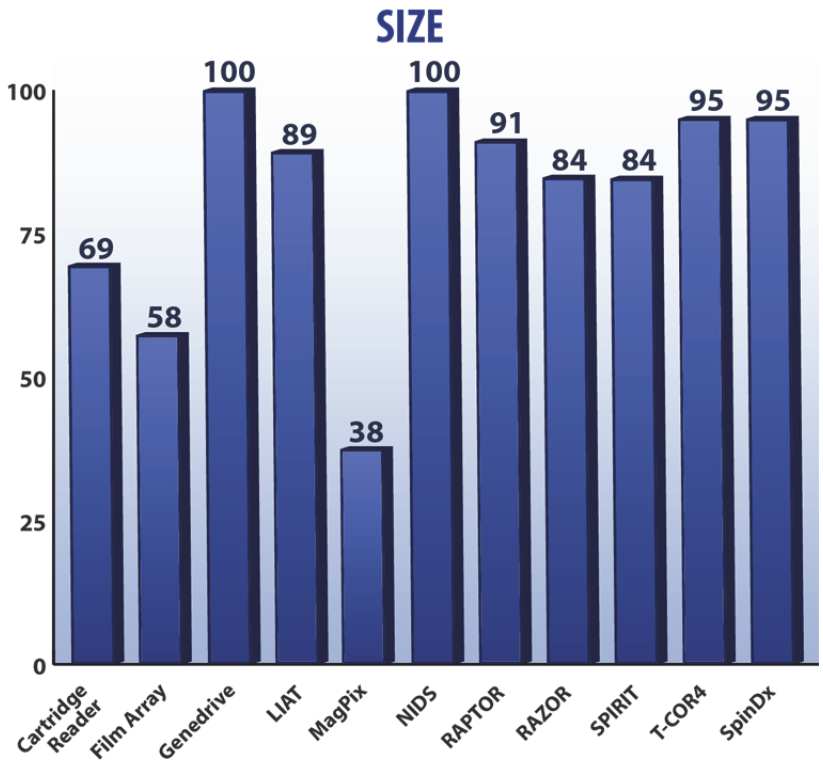


Figure 16. Comparison of Size scores

Power

The Power attribute noted (1) the ability of a system to run by battery power and (2) the anticipated power draw (in Watts) of the instrument. The high scores in this category denoted instruments that were battery-powered, as this was weighted high for end-users in field situations. The MAGPIX and FilmArray systems scored lowest for Power attribute because they do not utilize a battery and draw up to 900 Watts.

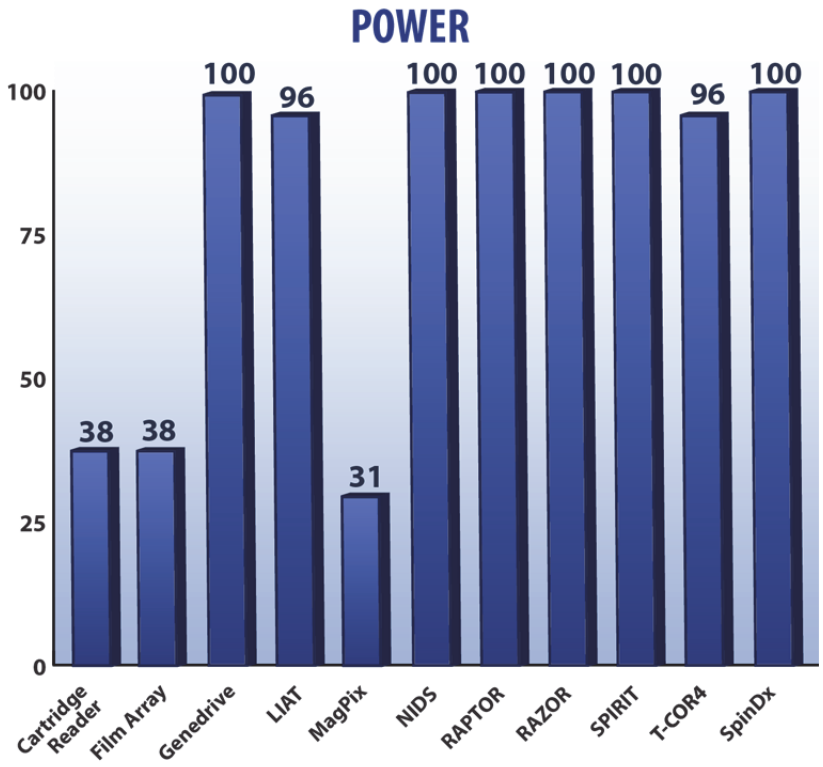


Figure 17. Summary of Power scores

DISCUSSION

Assay Design

Assay design is a parameter that was difficult to control across the various platforms evaluated in this study. Materials from the CRP were made available to each performer so that standardized assays could be used, but this was often not practical for all participants. Targets were chosen to encompass a wide spectrum of etiological agents including bacteria, viruses, and toxins to fully assess the devices' utility in detecting a broad range of targets. Manufacturers, such as ANP, had existing assays for their hand-held immunoassay cartridges and NIDS SAR III reader that covered the agents chosen for this evaluation. Other manufacturers, such as BioFire Diagnostics, had COTS assays for the RAZOR EX that did not detect all of the chosen agents; and, because of their closed architecture, an assay could not quickly be developed. On the other hand, companies including Mesoscale Diagnostics and Epistem created custom assays specifically for this study using the materials provided. As an added complication to controlling assay design, two complementary technologies were evaluated: direct immunological detection and nucleic-acid amplification. Throughout the evaluation, efforts were made to directly compare detection across all platforms regardless of the technical divergence. Rather than reporting merely the concentration of agent required for detection, the total amount of material required was reported, and in the case of Epistem and Tetracore, amount of purified DNA was converted to amount of agent.

Target Choice

Target choice, especially for the bacterial pathogens, has a significant influence on assay sensitivity and specificity. It has become common for assay developers to target both plasmids and chromosomal loci in multiplex assays. This provides organism identification as well as confirmation of episomal virulence factors simultaneously. Plasmids are usually found in multiple copies per cell, depending upon their size and nature of their replication genetics. Targeting such high-copy number loci can provide more consistent and sensitive detection, especially when trace contamination is present. However, plasmids are often shared between species through horizontal gene transfer, which can lead to equivocal results and loss of specificity. There is an inherent trade-off in terms of sensitivity and specificity when using either chromosomal or plasmid targets without multiplex, multi-locus confirmation. Of the technologies evaluated, the BioFire Diagnostics FilmArray was the only device for which multiplex assays amplifying more than one target per agent were available.

Direct detection of toxins represents a challenge for molecular methods, relying on residual DNA to provide signal for detection. Unfortunately, only the crudest of toxin preparations would be expected to reliably retain a molecular signature and the physical characteristics of the two classes of macromolecules (protein and nucleic acids) and the physical methods used to purify them are quite disparate. In this evaluation, three separate PCR detection systems (BioFire RAZOR EX, BioFire Film Array, and Tetracore T-COR 4) were unable to detect the *Botulinum* A toxin reference material. The only system not running an immunoassay to detect the toxin was the Epistem Genedrive which used a novel fluorogenic activity assay with a synthetic labeled peptide target. Hybrid detection systems such as the Luminex MAGPIX/xMAP technologies allow for detection of both molecular and immunological targets although only the immunoassay component of this system was evaluated in this trial.

Cross Platform Comparisons

As noted above, assay constraints (product specific assays/gaps in assay availability for some platforms) and systems with and without on-board sample preparation made comparisons of overall system sensitivity difficult. To compensate for this lack of controlled testing, every effort was made to ascertain the sensitivity of each platform (hardware, software, and assay) and present the data in a meaningful manner for acquisition managers. To this end, each set of results is presented in terms of target concentration (CFU/mL, PFU/mL, or ng/mL), absolute quantity per reaction (CFU, PFU, mass, or GE), and,

when available, assay target in order to control for internal/external sample preparation and disparate volumes used per system run. All certified reference materials (organisms, toxins, and DNA/RNA) were provided by the CRP, and, where possible, CRP assays were employed.

In general, products fell into two separate categories of maturity. Most of the systems had high technology readiness and represented FDA approved COTS and/or deployed DoD technologies. A minority subset of platforms was more developmental in nature at much lower technology readiness, which prevented complete or, in some cases, any testing of proposed capabilities. While not ready for formal acquisition programs (as indicated by the testing results), this should not preclude these less mature systems from being reevaluated after additional development has taken place to be considered for future programs. Biological detection methodologies are evolving very quickly, especially in the miniaturization of immunoassays and the speed and detection of nucleic acid amplification. By evaluating less mature systems, a technology readiness assessment allows an initial glimpse at emerging and future technologies and will provide acquisition managers with a point of reference to gauge improvements in performance.

While every effort was made to benchmark hardware functionality and sensitivity, it was impossible to completely decouple hardware performance from assay performance. The fact that some platforms have closed architectures that cannot be quickly or easily adapted to third-party assays does represent a weakness compared to others that can quickly adapt and optimize new content. *It is important for acquisition managers to understand that validity and performance of assays is in no way related to the performance of the underlying hardware systems, and that both should be evaluated independently whenever possible.*

In most cases, overall system sensitivity was at least slightly less than advertised by manufacturers. This is likely attributable by the lack of uniform reference materials across the diagnostics and biodefense industry space and variability of testing materials independently prepared by a large number of performers. However, each system was evaluated with the same lots of reference materials (prepared where possible and appropriate under ISO Guide 34) with testing performed in as ISO 17025-reference laboratory. While it is possible to dispute minor differences in results between a manufacturer's laboratory evaluation with those presented here, the relative differences in performance between platforms in this evaluation are defensible and suitable to support early-phase acquisition decision-making.

Throughput and Data Density

The technologies evaluated span a wide range of throughput and data density, going from single sample, singleplex detection to 96-well plate format with highly multiplexed detection. Most of the systems tested fall into the middle of this performance spectrum, providing intermediate throughput with more flexible assay and use-case options. Cost, processing time, overall throughput, and veracity of results are all important criteria for instrument selection to match specific Concept of Operations (CONOPS).

Sample to Answer

One recent push for recent technology development is the advent of "sample-to-answer" systems that contain on-board sample preparation and can provide a rapid, automated diagnostic result. Most of the devices tested in this evaluation were examined with this push in mind and were challenged with whole organism regardless of whether they had on-board sample preparation capabilities. The exceptions to this were the Tetracore T-COR 4 and Epistem Genedrive, both of which were tested with purified nucleic acids. The more advanced systems were able to provide FDA-approved multiplex syndromic panel

detection for respiratory/gastrointestinal infections and biological threat agents. This capability is both impressive and important for routine clinical applications, but at this phase of development has a high-cost per sample, low throughput (less than twenty samples per eight-hour shift), and fixed assay content.

Batch processing

Higher throughput and higher multiplex systems provide another facet of detection and diagnostic capabilities. As a rule, such systems require a higher level of trained staff, additional ancillary equipment for sample preparation/processing, and more sophisticated laboratory resources to employ. Straddling the gap between research applications and FDA-approved clinical use, they provide the most flexible technology base for emerging infectious disease threats with the lowest per-sample costs. By combining high levels of multiplexing with superior through-put, these systems generate the highest data density and support processing of large numbers of samples and multi-locus confirmatory testing.

Study Limitations

The primary purpose of this study was to evaluate the sensitivity of biological detection and diagnostic equipment, by verifying vendor claims and providing baseline comparisons between platforms. As discussed, it was not possible in all cases to eliminate other variables such as varying assay content and sample processing capabilities. Data was presented in a variety of formats to allow direct comparisons where possible, caveating differences of assay targets, sample volume utilization, and other confounding parameters.

Assay specificity was not addressed in a systematic manner, only evaluating a total of five viral, bacterial, and protein toxin targets for which assays were available. No near-neighbor organisms; pathogens causing “look-alike” disease; host, vector, environmental samples; or wide biodiversity panels were tested looking for clinical or environmental cross reactivity. Any system or assay testing that resulted in a false positive result with such a small testing cohort should be scrutinized for assay or instrument design flaws that would need to be addressed before fielded use.

No specific recommendations are suggested to match technologies to CONOPS. In each case, system capabilities, utility, and potential performance limitations have been documented to enable procurement managers, end users, and subject matter experts to use dependable test data to provide the best and most appropriate equipment for specific uses.

SUMMARY

Given the broad range of requirements for diagnostic and environmental testing, there is no single technology that provides a comprehensive solution for all needs. Routine, low-throughput screening of known threats is often most cost-effectively handled with rapid, low data density testing formats. However, epidemic/pandemic outbreaks, wide area-contaminations, and emerging biological threats require scalable technologies with the ability to scan a wider array of threats.

High consequence actions such as use of medical countermeasures or evacuation of critical infrastructure require a high burden of proof before implementation. Singleplex detection of biological threats is generally not considered to be sufficient to meet the burden of proof required for such actions, nor to rule out a potentially catastrophic threat for fear that a false-negative result will miss a new or genetically diverse existing threat.

A well-rounded suite of capabilities is required to meet the known scenarios for biological surveillance and defense. It is important that when specific applications and CONOPS are considered, that appropriate capabilities are matched in terms of costs, throughput, data density, and flexibility of architecture.

Appendix A: Technology Readiness Assignments

Technology Readiness Level (TRL)	Definition	ECBC Biosensors Test Bed Study TRL Interpretations	Department of Defense Technology Readiness Assessment Guidance Descriptions
1	Basic principles observed and reported.	Published research has identified the principles that underlie this technology. Basic scientific research has started to be translated into applied research and development. Examples include scientific literature and paper studies of a biological identifier technology's basic properties.	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.
2	Technology concept and/or application formulated.	Basic biological identification technology principles have been expressed in practical applications. Applications are speculative, and there may be no proof or detailed analysis to support the assumptions. Examples include technologies that are limited to analytical studies.	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.
3	Analytical and experimental critical function and/or characteristic proof of concept.	Active research and development, including analytical and laboratory studies to validate the analytical predictions of separate elements of the biological identifier technology. Examples include components that measure parameters of interest but are not yet integrated into or representative of the final system.	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.
4	Component and/or breadboard validation in a laboratory environment.	Basic biological identification technology components are integrated to establish feasibility. Non-optimized components are assembled and operated together. Laboratory testing shows capability to identify biological warfare agent (BWA) surrogates and/or biological simulants. Examples include integration of some components and "ad hoc" hardware in the laboratory.	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.
5	Component and/or breadboard validation in a relevant environment.	Basic technology components are integrated with supporting elements to function as a biological identifier in a simulated environment. Fidelity of the technology components is very high. System successfully shows capability to identify BWA surrogates or biological simulants. Initial assay development and sensitivity testing occurs.	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.
6	System model or prototype demonstration in a relevant environment.	Biological identifier system is well advanced beyond prototype and demonstrates technology's readiness. System and individual components are tested in a relevant environment for successful BWA surrogate or biological simulant identification. Optimization of assay performance with system.	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.

7	System prototype demonstration in an operational environment.	Biological identifier is at the operational stage and is a commercial-off-the-shelf (COTS) item. Major system components and software are in their final design. System operation is demonstrated in laboratory and field with BWA surrogates and/or biological stimulants and by field operators.	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).

Appendix B: Bio Identifier Assessment Tables

FilmArray (1 of 2)																
Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	500	7	70	5000	7	70	56
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	50	9	90	5	10	100	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	Unknown	0	0	1000	7	70	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	1000	7	70	1000000	4	40	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	N/A	0	0	0	0	0	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	500	7	35	5000	7	35	54
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	50	9	45	50	9	45	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	N/A	0	0	1000	7	35	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	1000	7	35	1000000	4	20	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	N/A	0	0	0	0	0	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	17	7	63	17	7	63	72
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	17	10	20	3	8	16	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	1	1	8	1	1	8	10
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	1	1	Company designed	1	1	8	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	1	0	0	1	0	0	0
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	0	10	20	0	10	20	57
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	5	9	72	2	10	80	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	60	1	9	65	1	9	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					Yes. Sample is mixed with a buffer prior to addition to assay pouch.						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					About 70 minutes including sample prep, running the pouch, and data interpretation.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	20	3	30	20	3	30	58
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5	0 <1.5 sq. ft.	10	90	1.1	9	81	1.1	9	81	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					Logistical footprint is approximately 4.7 sq. ft.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					Ancillary equipment weight is approximately 5 lbs.						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	No	0	0	No	0	0	38
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	90-200	9	45	77	10	50	

FilmArray (2 of 2)																
Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					All maintenance is performed by the company.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					Final report exportable as .XPS only.						
	Consumables	Info Only	Storage area required for consumables		*Informational only*					Assays shipped in kit of 6 assay pouches, less than 1 sq. ft. storage space required per kit.						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					None. Room temperature storage.						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Approximately 1 mL total waste remaining in sample and rehydration syringes. Note: Canula is stainless steel. Vendor recommends treating syringe and canula as sharps waste.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$49,500.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$49,500.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					$1,100.00						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service as necessary by BioFire.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Informational only*					Final report exportable as .XPS only.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					No. Assays are self contained. Recommend decontaminating sample inlet.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 7.						
															GRAND TOTAL	48

RAZOR (1 of 2)

Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	1000	7	70	13000	5	50	**24**
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	100	8	80	1000	7	70	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	N/A	0	0	No assay	0	0	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	1000	0	0	No assay	0	0	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	N/A	0	0	No assay	0	0	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	1000	7	35	26000	5	25	**24**
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	100	8	40	2600	7	35	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	N/A	0	0	No assay	0	0	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	N/A	0	0	No assay	0	0	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	N/A	0	0	No assay	0	0	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	10	5	45	10	5	45	**43**
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	1	1	2	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	1	1	8	1	1	8	**10**
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	1	1	8	1	1	8	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	1	0	0	1	0	0	**0**
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	5	9	18	5	9	18	**71**
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	5	9	72	5	9	72	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	30	5	45	30	5	45	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					Yes. Sample is diluted into Sample Dilution solution prior to loading pouch.						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					Result automatically displayed on LCD screen.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	11	7	70	11	7	70	**84**
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	0.31	10	90	0.31	10	90	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					None required.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					N/A						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	Yes	10	80	Yes	10	80	**100**
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	96	10	50	96	10	50	

RAZOR (2 of 2)																
Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					All maintenance is performed by the company.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					None						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Each kit measures 7.125 x 7.25 inches (0.36 sq. ft.).						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					None. Room temperature storage.						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Approximately 1mL liquid remains in syringes used for loading assay. Assay pouch is self-contained. Sample dilution vial contains approximately 5mL liquid.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$38,500.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$38,500.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					$200.00						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service by BioFire as necessary.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Informational only*					Results viewed on screen. Results can be downloaded using a proprietary software and laptop.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					External decontamination recommended.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 7						
															GRAND TOTAL	41

Genedrive (1 of 2)

Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	4200	6	60	2400000	4	40	
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	7200	6	60	18,000	5	50	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	6900	6	60	2900000	4	40	**38**
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	10000	5	50	No assay	0	0	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	No data	0	0	10 ng/mL	6	60	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	4200	6	30	N/A	0	0	
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	7200	6	30	N/A	0	0	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	6900	6	30	N/A	0	0	**0**
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	10000	5	25	N/A	0	0	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	No data	0	0	N/A	0	0	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	4	2	18	1	0	0	
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	1	1	2	1	1	2	**2**
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	3+	10	80	1	1	8	
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	Self	10	80	Company designed	5	40	**30**
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	3	2	18	3	2	18	**20**
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	0	10	20	0	10	20	
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	15	8	64	15	8	64	**54**
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	60	2	18	60	2	18	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					Not as advertised, but system was tested with purified DNA.						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					About 70 minutes including sample preparation, running the assay, and data interpretation.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	1.2	10	100	1.2	10	100	
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	0.5	10	90	0.5	10	90	**100**
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					1 square foot						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					0.5 pound						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	Yes	10	80	Yes	10	80	
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	100	10	50	100	10	50	**100**

Genedrive (2 of 2)															
Category	**Attribute**	**Attribute Weight (1 to 10)**	**Description**	**Unit of Measure**	**Grading Scale**			**Ideal Instrument**		**Vendor Supplied Information**			**Hardware Testing**			**Overall Score %**
					Low (1-3)	**Medium (4-7)**	**High (8-10)**	**Grade**	**Score (Wt. x Grade)**	**Claim**	**Grade**	**Score (Wt. x Grade)**	**Result**	**Grade**	**Score (Wt. x Grade)**	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					All maintenance is performed by the company.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					As advertised: 2B BlackBio BlackLight paper, 1mm biopsy punch, GE Ready-to-Go PureTaq PCR Beads. As tested, did not used paper or punch.						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Each kit required <1 square foot storage space.						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					As advertised: Room temperature As tested: Primers required refrigerator/freezer.						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					None						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$4,000.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$4,000.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					Estimated to be $85 dependent on volume purchased.						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service as necessary by Epistem or replacement unit provided.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Score = 1 for 1 target*					As advertised, no exportable data. As tested, data was exported as .jpg, .xls, and .txt files.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					External only						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 5.						
															GRAND TOTAL	**42**

Liat (1 of 2)

Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	9.8	10	100	1000	8	80	**64**
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	5.4	10	100	6.5	10	100	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	250	8	80	2500	7	70	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	2100	7	70	2100	7	70	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	N/A	0	0	0	0	0	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	9.8	10	50	1000	8	40	**60**
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	5.4	10	50	6.5	10	50	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	250	8	0	25000	6	30	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	2100	7	35	21000	6	30	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	N/A	0	0	0	0	0	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	2	1	9	2	1	9	**10**
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	1	1	2	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	3+	8	64	1	1	8	**10**
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	10	10	80	Company designed	1	8	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer.	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	1	0	0	1	0	0	**0**
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	2	9	18	2	9	18	**80**
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	0	10	80	0	10	80	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	20	6	54	20	6	54	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					None. Room temperature storage.						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					1 minute						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	8.3	8	80	8.3	8	80	**89**
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	<1	10	90	<1	10	90	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					No ancillary equipment.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					No ancillary equipment.						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	Yes	10	80	Yes	10	80	**96**
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	129	9	45	129	9	45	

Liat (2 of 2)

Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					All maintenance is performed by the company.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					None						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Less than 1 sq. ft. refrigeration area required per assay.						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					Assay tube kits must be stored at 4°C.						
	Open Architecture	Info Only	Multiple sources of consumables or self-designed assays	Reagent sources (List)	*Informational only*					No						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Assays are self contained. No waste stream other than assay tube.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$25,000.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$25,000.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					Market value not set. Estimated cost per assay is $65.						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					To be determined.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Informational only*					System saves files as .flt, .asy, .xml, .lddg, and .rst extensions. All files are readable with standard text editor, excel, or html display. System shipped with data recovery thumb drive.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external ?)	*Informational only*					No. Assays are self contained. Recommend decontaminating sample inlet.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 7.						
															GRAND TOTAL	**58**

T-Cor 4 (1 of 2)

Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	No data			100	8	80	**30**
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	No data			250	7	70	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	No data			No assay	0	0	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	No data			No assay	0	0	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	No data			No assay	0	0	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	No data			N/A	0	0	**0**
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	No data			N/A	0	0	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	No data			N/A	0	0	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	No data			N/A	0	0	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	No data			N/A	0	0	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	1	0	0	1	0	0	**2**
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	1	1	2	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	3+	10	80	3+	10	80	**100**
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	Self	10	80	Self	10	80	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	4	2	18	4	2	18	**20**
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	0	10	20	0	10	20	**41**
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	30	6	48	30	6	48	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	45	1	9	45	1	9	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					Yes. Qiagen mini kit used with each sample before analyzing.						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					About 70 minutes including sample preparation, running the assay, and data interpretation.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	6.2	9	90	6.2	9	90	**95**
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	<1	10	90	<1	10	90	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					1 sq. ft.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					1 lb.						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	Yes	10	80	Yes	10	80	**96**
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	120	9	45	120	9	45	

T-Cor 4 (2 of 2)																
Category	**Attribute**	**Attribute Weight (1 to 10)**	**Description**	**Unit of Measure**	**Grading Scale**			**Ideal Instrument**		**Vendor Supplied Information**			**Hardware Testing**			**Overall Score %**
					Low (1-3)	**Medium (4-7)**	**High (8-10)**	**Grade**	**Score (Wt. x Grade)**	**Claim**	**Grade**	**Score (Wt. x Grade)**	**Result**	**Grade**	**Score (Wt. x Grade)**	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					All maintenance is performed by the company.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					TCOR Centrifuge						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Each kit required <1 sq. ft. storage space.						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					Room temperature						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					None						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$16,000.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$16,000.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					$12.00						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service as necessary by Tetracore.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Score = 1 for 1 target*					Runs only saved if used with a PC. TCOR uses proprietary software not reviewed in the evaluation.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					External only.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 7.						
															GRAND TOTAL	**44**

NIDS (1 of 2)																
Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	1.00E+06	4	40	10,000,000	3	30	**30**
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	2.50E+05	5	50	2,500,000	4	40	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	1.00E+06	2	20	>100,000,000	1	10	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	1.00E+08	2	20	##########	1	10	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	50ng/mL	6	60	50 ng/mL	6	60	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	1.00E+06	4	20	10,000,000	3	15	**26**
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	2.50E+05	5	25	2,500,000	4	20	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	1.00E+06	2	10	>100,000,000	1	5	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	1.00E+08	2	10	##########	1	5	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	50 ng/mL	5	25	500 ng/mL	4	20	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	5	3	27	5	3	27	**26**
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	1	1	2	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	1	1	8	1	1	8	**30**
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	Company	5	40	Company	5	40	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	1	0	0	1	0	0	**0**
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	0	10	20	0	10	20	**86**
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	0	10	80	0	10	80	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	16	7	63	16	7	63	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					No						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					About 16 minutes including time to read assay strip.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	1.6	10	100	1.6	10	100	**100**
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	0.08	10	90	0.08	10	90	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					No ancillary equipment required.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					None required.						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	Yes	10	80	Yes	10	80	**100**
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	5	10	50	5	10	50	

NIDS (2 of 2)																
Category	**Attribute**	**Attribute Weight (1 to 10)**	**Description**	**Unit of Measure**	**Grading Scale**			**Ideal Instrument**		**Vendor Supplied Information**			**Hardware Testing**			**Overall Score %**
					Low (1-3)	**Medium (4-7)**	**High (8-10)**	**Grade**	**Score (Wt. x Grade)**	**Claim**	**Grade**	**Score (Wt. x Grade)**	**Result**	**Grade**	**Score (Wt. x Grade)**	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					Approximately 12 hours/year for cleaning the SARIII detector glass 1-2 times/month.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					None						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Bag of 50 assays takes up approximately 1 sq. ft.						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					Room temperature						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Assay coupon disposable. No liquid waste as all liquid is absorbed into coupon.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$6,500.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$6,500.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					$45 per 5-plex assay						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					None expected; SARIII service done by manufacturer as necessary.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Informational only*					Data files can be downloaded to a computer and stored in a proprietary database.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					None. Assays are self-contained. Recommend decontaminating reader surface. Bottom of unit is removable for decontamination of assay insertion area and reader lens.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 7.						
															GRAND TOTAL	**48**

MagPix (1 of 2)

Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	none	N/A	N/A	100,000	6	60	40
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	none	N/A	N/A	100,000	6	60	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	none	N/A	N/A	10,000,000	3	30	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	none	N/A	N/A	100,000,000	2	20	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	none	N/A	N/A	1,000	3	30	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	none	N/A	N/A	10,000,000	3	15	24
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	none	N/A	N/A	1,000	4	20	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	none	N/A	N/A	Could not test	0	0	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	none	N/A	N/A	1,000,000,000	2	10	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	none	N/A	N/A	1,000	3	15	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	50	10	90	50	10	90	84
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	none	N/A	N/A	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	3+	10	80	3+	10	80	100
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	Self	10	80	Self	10	80	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	96	10	90	96	10	90	100
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	15	3	6	15	3	6	3
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	115	0	0	115	0	0	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	115	0	0	115	0	0	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					There are multiple manual sample and reagent manipulation steps.						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					Raw data must be imported into Excel and interpreted. Software has limited functionality to automatically score qualitative results. Approximately 15 required.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	39	0	0	39	0	0	38
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	1	8	72	1	8	72	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					Approximately 6 sq. ft.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					20 lbs.						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	No	0	0	No	0	0	31
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	240	8	40	240	8	40	

MagPix (2 of 2)																
Category	**Attribute**	**Attribute Weight (1 to 10)**	**Description**	**Unit of Measure**	**Grading Scale**			**Ideal Instrument**		**Vendor Supplied Information**			**Hardware Testing**			**Overall Score %**
					Low (1-3)	**Medium (4-7)**	**High (8-10)**	**Grade**	**Score (Wt. x Grade)**	**Claim**	**Grade**	**Score (Wt. x Grade)**	**Result**	**Grade**	**Score (Wt. x Grade)**	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					13 hours for weekly maintenance run of approximately 15 minutes.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					Magnetic bead separator, computer, monitor, plate shaker, multi-channel pipets						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Less than 1 sq. ft. refrigeration area required.						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					Most consumables must be stored at 4°C.						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Approximately 400uL per sample or 4mL per full 96-well plate.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$24,000.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$24,000.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					$2-20 varies with batch size and antibody used.						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service as necessary by Luminex; 12-Month Preventative Maintenance kit is $150.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Informational only*					Tab delimited						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					Yes. Internal components exposed to test agent.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 7.						
															GRAND TOTAL	**44**

Cartridge Reader (1 of 2)																
Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	6.68E+08	2	20	100,000	6	60	42
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	3.01E+09	1	10	100,000	6	60	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	1.50E+08	2	20	10,000,000	3	30	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	1.10E+10	0	0	100,000,000	2	20	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	70 pg/mL	9	90	100 ng/mL	4	40	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	6.68E+08	2	10	100,000	6	30	38
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	3.01E+09	1	5	100,000	6	30	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	1.50E+08	2	10	100,000,000	2	10	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	1.10E+10	0	0	100,000,000	2	10	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	70 pg/mL	9	45	10000 ng/mL	3	15	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	5	2	18	12	5	45	43
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	1	1	2	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	1	1	8	1	1	8	30
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	Company	5	40	Company	5	40	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	1	0	0	1	0	0	0
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	0	10	20	0	10	20	81
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	0	10	80	0	10	80	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	30	6	54	30	6	54	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					No						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					About 30 minutes.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	13	5	50	13	5	50	69
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	0.68	9	81	0.68	9	81	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					No ancillary equipment required.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					No ancillary equipment required.						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	No	0	0	No	0	0	38
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	18	10	50	18	10	50	

Cartridge Reader (1 of 2)																
Category	**Attribute**	**Attribute Weight (1 to 10)**	**Description**	**Unit of Measure**	**Grading Scale**			**Ideal Instrument**		**Vendor Supplied Information**			**Hardware Testing**			**Overall Score %**
					Low (1-3)	**Medium (4-7)**	**High (8-10)**	**Grade**	**Score (Wt. x Grade)**	**Claim**	**Grade**	**Score (Wt. x Grade)**	**Result**	**Grade**	**Score (Wt. x Grade)**	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					Maintenance performed by MSD.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					None						
	Consumables	Info Only	Storage area required for consumables		*Informational only*					Assays shipped in individual foil pouches. Each assay is <1 sq. ft.						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					Assays must be refrigerated.						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Liquid is contained within the assay cartridge.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$85000 in current configuration.						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$85000 in current configuration.						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					To be determined.						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service done by MSD as necessary.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Informational only*					Data is stored on device and can be downloaded to an SD card in .cvs format. Initially, data had to be transferred to an excel spreadsheet for analysis, but updated firmware displays results summary on screen.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					No. Assays are self-contained.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 6.						
															GRAND TOTAL	**45**

Raptor (1 of 2)

Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	5.00E+04	6	60	>5,000,000	1	10	**14**
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	No Claim	0	0	50,000,000	3	30	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	1.00E+05	6	60	>100000000	1	10	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	N/A	0	0	N/A	0	0	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	10ng/mL	7	70	> 10,000 ng/ml	2	20	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	1.00E+06	4	20	>5,000,000	1	5	**8**
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	2.50E+05	5	25	50,000,000	3	15	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	1.00E+06	4	20	N/A	0	0	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	N/A	0	0	N/A	0	0	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	50 ng/mL	6	30	>ug/mL	0	0	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	4	2	18	4	2	18	**18**
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	4	4	8	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	1	1	8	1	1	8	**30**
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	Company	5	40	Company	5	40	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer.	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	1	0	0	1	0	0	**0**
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	0	10	20	0	10	20	**76**
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	0	10	80	0	10	80	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	15	7	63	28	5	45	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					No						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					Approximately 28 minutes, includes time required for baseline correction (required for a new assay coupon).						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	12.3	5	50	12.3	10	100	**91**
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	1	8	72	1	8	72	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	square feet	*Informational only*					Less than 0.5 sq. ft.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					About 1 lb.						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	Yes	10	80	Yes	10	80	**100**
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	30	10	50	30	10	50	

Raptor (2 of 2)																
Category	**Attribute**	**Attribute Weight (1 to 10)**	**Description**	**Unit of Measure**	**Grading Scale**			**Ideal Instrument**		**Vendor Supplied Information**			**Hardware Testing**			**Overall Score %**
					Low (1-3)	**Medium (4-7)**	**High (8-10)**	**Grade**	**Score (Wt. x Grade)**	**Claim**	**Grade**	**Score (Wt. x Grade)**	**Result**	**Grade**	**Score (Wt. x Grade)**	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					26 hours/year for weekly maintenance run.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					A/C Adapter, battery charger						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Each kit measures 2.5 x 3 inches (0.05 sq. ft.).						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					Refrigeration						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Approximately 10mL per test.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$49,500.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$49,500.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					$150 per custom cartridge (re-usable).						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service as necessary by Research International.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Informational only*					Proprietary software required to download files.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					Yes. Internal system components are exposed to test agent.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 6.						
															GRAND TOTAL	**37**

SpinDx (1 of 2)																
Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	N/A	0	0	0	0	0	0
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	N/A	0	0	0	0	0	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	N/A	0	0	0	0	0	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	N/A	0	0	0	0	0	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	N/A	0	0	0	0	0	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	N/A	0	0	0	0	0	0
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	N/A	0	0	0	0	0	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	N/A	0	0	0	0	0	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	N/A	0	0	0	0	0	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	N/A	0	0	0	0	0	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	20	8	72	2	1	9	10
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	1	1	2	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	1	1	8	1	1	8	30
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	Company	5	40	Company	5	40	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	15	4	36	15	4	36	40
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	0	10	20	0	10	20	78
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	20	7	56	20	7	56	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	15	8	72	15	8	72	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					Yes. Sample is diluted into Sample Dilution solution prior to loading pouch.						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					Result automatically displayed on LCD screen.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	4	9	90	4	9	90	95
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	0.2	10	90	0.2	10	90	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					None required.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					N/A						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	Yes	10	80	Yes	10	80	100
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	~200	8	40	25	10	50	

SpinDx (2 of 2)																
Category	**Attribute**	**Attribute Weight (1 to 10)**	**Description**	**Unit of Measure**	**Grading Scale**			**Ideal Instrument**		**Vendor Supplied Information**			**Hardware Testing**			**Overall Score %**
					Low (1-3)	**Medium (4-7)**	**High (8-10)**	**Grade**	**Score (Wt. x Grade)**	**Claim**	**Grade**	**Score (Wt. x Grade)**	**Result**	**Grade**	**Score (Wt. x Grade)**	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					All maintenance is performed by the company.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					None						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Each kit measures 7.125 x 7.25 inches (0.36 sq. ft.).						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					None. Room temperature storage.						
	Open Architecture	Info Only	Multiple sources of consumables or self-designed assays	Reagent sources (List)	*Informational only*					No						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Approximately 1mL liquid remains in syringes used for loading assay. Assay pouch is self-contained. Sample dilution vial contains approximately 5mL liquid.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$38,500.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$38,500.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					$200.00						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service as necessary by Sandia National Laboratory staff.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Score = 1 for 1 target*					Results viewed on screen. Results can be downloaded using a proprietary software and laptop.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					External decontamination recommended.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 4.						
															GRAND TOTAL	**34**

Spirit (1 of 2)

Category	Attribute	Attribute Weight (1 to 10)	Description	Unit of Measure	Grading Scale			Ideal Instrument		Vendor Supplied Information			Hardware Testing			Overall Score %
					Low (1-3)	Medium (4-7)	High (8-10)	Grade	Score (Wt. x Grade)	Claim	Grade	Score (Wt. x Grade)	Result	Grade	Score (Wt. x Grade)	
Singleplex Target Identification	Singleplex Sensitivity - Gram+	10	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	1000	7	70	13000	5	50	**24**
	Singleplex Sensitivity - Gram-	10	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	100	100	8	80	1000	7	70	
	Singleplex Sensitivity - DNA Virus	10	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	N/A	0	0	No assay	0	0	
	Singleplex Sensitivity - RNA Virus	10	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	100	1000	0	0	No assay	0	0	
	Singleplex Sensitivity - Toxin	10	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	100	N/A	0	0	No assay	0	0	
Multiplex Target Identification	Multiplex Sensitivity - Gram+	5	Limit of detection (Gram + bacteria), *B. anthracis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	1000	7	35	26000	5	25	**24**
	Multiplex Sensitivity - Gram-	5	Limit of detection (Gram - bacteria), *Y. pestis*	cfu/ml	Bacteria: 10e7-10e9	Bacteria: 1000-10e6	Bacteria: 1-100	10	50	100	8	40	2600	7	35	
	Multiplex Sensitivity - DNA Virus	5	Limit of detection (DNA Virus), Vaccinia	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	N/A	0	0	No assay	0	0	
	Multiplex Sensitivity - RNA Virus	5	Limit of detection (RNA Virus), VEE	pfu/ml	Virus: 10e7-10e9	Virus: 1000-10e6	Virus: 1-100/mL	10	50	N/A	0	0	No assay	0	0	
	Multiplex Sensitivity - Toxin	5	Limit of detection (Toxin), Botulinum Type A Toxin	mass/mL	Toxins: ug/mL	Toxins: ng/mL	Toxins: pg/mL	10	50	N/A	0	0	No assay	0	0	
Multiplex Capability	Multiplex	9	Number of reportable agents per run	# of agents per run	1 = 0 score; 2-7	8-19	20-34+	10	90	10	5	45	2	1	9	**10**
	Multiplex	2	Number of individual targets per test	# individual targets per test	Score = 1 for 1 target	Score = 5 for 2 targets	Score =8+ for 3+ targets	10	20	10	9	18	1	1	2	
Assay Flexibility	Number of Assay Sources	8	Multiple sources of consumables or self-designed assays	# of sources	Score = 1 for 1 source	Score = 5 for 2 sources	Score =8+ for 3+ sources	10	80	3+	10	80	1	1	8	**30**
	Assay Development	8	New assay integration	Ease of acquisition	Score = 1 for Financially limiting	Score = 5 for Company designed	Score = 10 for Self designed	10	80	Company	5	40	Company	5	40	
Batch Size	Number of Samples	9	If looking for a single agent, the number of samples that can be processed at the same time by one analyzer	# of samples	1 = 0 score; 2-9	10-50	50+	10	90	1	0	0	1	0	0	**0**
Run Time	Boot Up	2	Time required for warm-up and calibration prior to sample analysis	Minutes	14+	6<14	0<6	10	20	5	9	18	5	9	18	**71**
	Sample Preparation Time	8	Time required from receipt of sample until it is ready to be analyzed	Minutes	35+	15<35	0<15	10	80	5	9	72	5	9	72	
	Analysis Time	9	Time required to provide a test answer after the analysis has been initiated	Minutes	35+	15<35	0<15	10	90	30	5	45	30	5	45	
	Sample Preparation	Info Only	Sample preparation required?	Yes/No	*Informational only*					Yes. Sample is diluted into Sample Dilution solution prior to loading pouch.						
	Total Analysis Time	Info Only	The duration of time to provide final identification of target from raw data	Minutes	*Informational only*					Result automatically displayed on LCD screen.						
Size	Weight	10	Approximate weight of instrument with power source	Pounds	24-36	8<24	2<8	10	100	11	7	70	11	7	70	**84**
	Instrument Size	9	Approximate footprint of instrument only in operational mode	Square feet	3.5+ sq. ft.	1.5<3.5 sq. ft.	0 <1.5 sq. ft.	10	90	0.31	10	90	0.31	10	90	
	Logistical Footprint	Info Only	Approximate footprint of instrument with ancillary equipment	Square feet	*Informational only*					None required.						
	Ancillary Equipment Weight	Info Only	Approximate weight of ancillary equipment (microcentrifuge, plate shaker, plate washer, laptop)	Pounds	*Informational only*					N/a						
Power	Power Requirements	8	Battery supplied	Yes/No	No = 0; Yes = 10			10	80	Yes	10	80	Yes	10	80	**100**
	Power Requirements	5	Expected power required	Watts	700+	301-700	0-300	10	50	45	10	50	45	10	50	

Spirit (2 of 2)																
Category	**Attribute**	**Attribute Weight (1 to 10)**	**Description**	**Unit of Measure**	**Grading Scale**			**Ideal Instrument**		**Vendor Supplied Information**			**Hardware Testing**			**Overall Score %**
					Low (1-3)	**Medium (4-7)**	**High (8-10)**	**Grade**	**Score (Wt. x Grade)**	**Claim**	**Grade**	**Score (Wt. x Grade)**	**Result**	**Grade**	**Score (Wt. x Grade)**	
Logistical Support	User Maintenance	Info Only	Time required for Preventative Maintenance	Hours per year	*Informational only*					All maintenance is performed by the company.						
	Ancillary Equipment	Info Only	List ancillary equipment requirement for operation of this instrument	List ancillary equipment	*Informational only*					None						
	Consumables	Info Only	Storage area required for consumables	Square feet	*Informational only*					Each kit measures 7.125 x 7.25 inches (0.36 sq. ft.)						
	Consumables Requirements	Info Only	Special conditions required for consumables (e.g., refrigeration, high humidity)	Conditions	*Informational only*					None. Room temperature storage.						
	Waste Management	Info Only	Approximate volume of waste streams	Approximate mL per test	*Informational only*					Approximately 1mL liquid remains in syringes used for loading assay. Assay pouch is self-contained. Sample dilution vial contains approximately 5mL liquid.						
Costs	Quoted Cost	Info Only	System cost as quoted or listed	$	*Informational only*					$38,500.00						
	Purchase Cost	Info Only	Actual system cost as purchased	$	*Informational only*					$38,500.00						
	Reagent Cost	Info Only	Reagent cost per sample	$	*Informational only*					$200 per sample chip (re-usable).						
	Service Cost	Info Only	Cost for yearly service	$	*Informational only*					Service as necessary by Seattle Sensors Systems.						
Compatibility/ Interchangeability	Interoperability	Info Only	Types of data files for export (e.g., ASCII, XML, tab delimited text)	File types (list)	*Score = 1 for 1 target*					Results viewed on screen. Results can be downloaded using a proprietary software and laptop.						
Usability	Decontamination Requirements	Info Only	Is decontamination required?	Yes/No (If Yes, internal and/or external?)	*Informational only*					External decontamination recommended.						
Maturity	Readiness Level	Info Only	Current Technical Readiness Level (TRL)	TRL	*Informational only*					Based on subject matter expert feedback, the TRL is 5.						
															GRAND TOTAL	**41**

Appendix C: System Evaluation Worksheets

GENERAL INFORMATION	
Instrument Name	FilmArray
Instrument Serial Number	FA2070
Firmware Version	Master: 1.3.4, Thermocycler: 1.3.27, Valve: 1.6.9, Protocol: 2
Is this a COTS Instrument?	Yes
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	40 (laptop, barcode reader, device, and pouch preparation assembly)
System Footprint (w" x d" x h")	10 x 15.5 x 6.5
Total footprint with ancillary equipment (w" x d" x h")	34 x 20 x 11 (including laptop, barcode reader, device, pouch loading station, cables, and mouse)
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Yes
Was additional equipment required to power on the device? If so, explain.	No
Comments: Manual is well written, easy to understand. Manual also has troubleshooting section and safety information.	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	No
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	System is shipped with laptop and barcode reader. Barcode reader is optional.
Were there any special power requirements?	No. 110V A/C
How much time was required to set up the instrument?	15 minutes
How long did training take?	45 minutes to read through manual
Did you call the company with questions prior to starting your first run?	Yes
Did the company have a dedicated tech support person that was able to help you?	N/A
Are any initial calibrations required? If so, insert a description and time required in Comments.	Barcode reader didn't function during pre-assessment. Required calibration using barcodes included in User Manual. Calibration procedure took approximately 30 seconds.
Comments: None	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
BioThreat Panel v2.4	Room Temp	6 months
Are there any reagents that require additional preparation by the end-user?	BioThreat Panel Kit contains all reagents required except dilution buffer. Kit contains array, array hydration buffer, sample buffer, syringes with canulas, and sample transfer pipette.	
Are there any reagents that were not supplied by the company?	Dilution buffer if samples will be diluted prior to assessment.	

GENERAL INFORMATION	
Instrument Name	RAZOR
Instrument Serial Number	EX4353
Firmware Version	rev06Jan05 2012
Is this a COTS Instrument?	Yes
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	11
System Footprint (w" x d" x h")	9.25 x 4.5 x 8
Total footprint with ancillary equipment (w" x d" x h")	N/A
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Yes
Was additional equipment required to power on the device? If so, explain.	No
Comments: None	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	No
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	N/A
Were there any special power requirements?	No
How much time was required to set up the instrument?	< 5 minutes
How long did training take?	45 minutes to read instruction manual and watch videos
Did you call the company with questions prior to starting your first run?	No
Did the company have a dedicated tech support person that was able to help you?	N/A
Are any initial calibrations required? If so, insert a description and time required in Comments.	No
Comments: None	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
Reagent Grade Water	18-25 C and 85% Humidity	N/A
Unknown Sample Bottle	18-25 C and 85% Humidity	N/A
The 10 Pouch (PATH-ASY-0061)	18-25 C and 85% Humidity	6 months
Are there any reagents that require additional preparation by end-user?	No	
Are there any reagents that were not supplied by the company?	No	

GENERAL INFORMATION	
Instrument Name	Genedrive
Instrument Serial Number	9f13f7f-95.6-647-i
Firmware Version	Unknown
Is this a COTS Instrument?	Yes—but is still in development
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	1.2
System Footprint (w" x d" x h")	5 x 7.5 x 5
Total footprint with ancillary equipment (w" x d" x h")	Requires up to an additional 100 in^2 workspace for assay set up and storage of ancillary equipment
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Battery packs were not delivered with units.
Was the training/operator manual included?	Yes
Was additional equipment required to power on the device? If so, explain.	A/C adapter or battery pack
Comments: There were difficulties in shipment of supplies from United Kingdom to Edgewood Chemical Biological Center. For example, batteries were held in customs causing a delay in delivery.	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	No. Everything is stored at room temperature.
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	2B BlackBio BlackLight Card, Assay cartridges with lyophilized primer/probes, Ready-To-Go PureTaq PCR Beads (GE), Biopsy punch, PCR Grade water, cartridge cap assemblies. As tested for assessment, cartridges were empty and primer/probes delivered separately as lyophilized products.
Were there any special power requirements?	No
How much time was required to set up the instrument?	Less than 5 minutes. Assay set up required about 15 minutes.
How long did training take?	About a 20 minute demonstration.
Did you call the company with questions prior to starting your first run?	No. Company had demonstrated use of the device and accessories.
Did the company have a dedicated tech support person that was able to help you?	Worked nearly exclusively with Director of Diagnostics.
Are any initial calibrations required? If so, insert a description and time required in Comments.	None
Comments: We requested engineering software from Epistem that allowed us to view data on the device. The software required a laptop. This would not be required for field use or laboratory assessment of full production model.	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
Assay Cartridges with Lyophilized Primer/Probes	Room Temperature	1 year
2B BlackBio BlackLight Paper	Room Temperature	Unknown
Ready-To-Go PureTaq PCR Beads	Room Temperature	Approximately 2 years
Separated Lyophilized Primer/Probes	Room Temperature	1 year
PCR Grade Water	Room Temperature	1 year
Are there any reagents that require additional preparation by the end-user?	When testing with separated lyophilized primer/probes, primer/probes were reconstituted with PCR grade water, stored at 4°C (short term)/ 20°C (long term)	
Are there any reagents that were not supplied by the company?	DNA purification kit for evaluation of system with purified DNA	

GENERAL INFORMATION	
Instrument Name	LIAT
Instrument Serial Number	M1-D-00065
Firmware Version	1.4.0
Is this a COTS Instrument?	Yes
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	8.3
System Footprint (w" x d" x h")	4.5 x 10 x 7.5
Total footprint with ancillary equipment (w" x d" x h")	N/A
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Yes
Was additional equipment required to power on the device? If so, explain.	No
Comments: N/A	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	Kept at 4°C
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	External battery
Were there any special power requirements?	No
How much time was required to set up the instrument?	10 minutes
How long did training take?	30 minutes to read instruction manual.
Did you call the company with questions prior to starting your first run?	No
Did the company have a dedicated tech support person that was able to help you?	No
Are any initial calibrations required? If so, insert a description and time required in Comments.	No
Comments: Product should be operated between 15°C and 32°C. Analyzer should be level, free of vibrations, and out of direct sunlight.	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
Assay Tubes	4°C	1 year
Are there any reagents that require additional preparation by the end-user?	No	
Are there any reagents that were not supplied by the company?	No	

GENERAL INFORMATION	
Instrument Name	T-Cor 4
Instrument Serial Number	XXX
Firmware Version	XXX
Is this a COTS Instrument?	Yes
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	6.2
System Footprint (w" x d" x h")	9 x 7.5 x 2.5
Total footprint with ancillary equipment (w" x d" x h")	N/A
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Yes
Was additional equipment required to power on the device? If so, explain.	No
Comments: None	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	No
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	Mini centrifuge
Were there any special power requirements?	No
How much time was required to set up the instrument?	< 5 minutes
How long did training take?	30 minutes to read instruction manual.
Did you call the company with questions prior to starting your first run?	No
Did the company have a dedicated tech support person that was able to help you?	N/A
Are any initial calibrations required? If so, insert a description and time required in Comments.	No
Comments: None	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
Rehydration Buffer	15-30°C	1 year
Test Sample Tubes in bag	15-30°C	1 year
Positive Control Tubes	15-30°C	1 year
Are there any reagents that require additional preparation by end-user?	No	
Are there any reagents that were not supplied by the company?	No	

GENERAL INFORMATION	
Instrument Name	NIDS
Instrument Serial Number	20110047
Firmware Version	V2.0.3.29
Is this a COTS Instrument?	Yes
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	~ 3.5
System Footprint (w" x d" x h")	2.5 x 4.75 x 4
Total footprint with ancillary equipment (w" x d" x h")	16 x 9.5 x 4
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Instruction manual included.
Was additional equipment required to power on the device? If so, explain.	Device has USB rechargeable battery pack that requires a computer or other USB power source.
Comments: None	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	No
Were the reagents received as required by the manufacturer	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	Laptop computer for device power and download of results.
Were there any special power requirements?	USB
How much time was required to set up the instrument?	<5 minutes excluding installation of optional computer software.
How long did training take?	Simple to learn. <10 minutes including sample preparation.
Did you call the company with questions prior to starting your first run?	No
Did the company have a dedicated tech support person that was able to help you?	N/A
Are any initial calibrations required? If so, insert a description and time required in Comments.	No
Comments: Device appears to discharge quickly when not connected to computer or computer in standby mode.	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
5-Plex 1 Cartridge	Room Temp	FAQ lists 2 years from date of manufacture (DOM). ANP says 1 year from receipt.
5-Plex 2 Cartridge	Room Temp	FAQ lists 2 years from DOM. ANP says 1 year from receipt
Are there any reagents that require additional preparation by end-user?	PBSTK, PBS, or other dilution buffers.	
Are there any reagents that were not supplied by the company?	PSBTK is available from company but not originally supplied.	

GENERAL INFORMATION	
Instrument Name	MagPix
Instrument Serial Number	MagPx12214702
Firmware Version	1.1.539
Is this a COTS Instrument?	4.2 Build 1324
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	38.5
System Footprint (w" x d" x h")	6.5 x 23x 16.5
Total footprint with ancillary equipment (w" x d" x h")	26 x 23 x 16.5
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Yes. Manual included on disc. Training videos included with software.
Was additional equipment required to power on the device? If so, explain.	A computer is required to control the system.
Comments: System was set up by Luminex personnel.	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	Refrigeration
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	Vortemp for plate incubations, vortexer, magnetic 96-well format base for bead trapping, magnetic bead trapper for 1.5mL microcentrifuge tubes, multi-channel micropipette, sonicator for preventive maintenance
Were there any special power requirements?	No
How much time was required to set up the instrument?	90 minutes
How long did training take?	240 minutes
Did you call the company with questions prior to starting your first run?	Yes. Discussed selection of bead regions and overall assay design.
Did the company have a dedicated tech support person that was able to help you?	Dealt directly with Sr. Field Application Specialist and/or Director of Government Business Development.
Are any initial calibrations required? If so, insert a description and time required in Comments.	System requires an initial calibration and performance verification which must be repeated weekly.
Comments: Suggested preventive maintenance is extensive including weekly, monthly, bi-annual, and annual operations.	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
PBS + 1 % BSA ("Block")	4°C	6 months – 1 year
PBS + 0.1 % Tween-20 ("Wash Buffer")	4°C	6 months – 1 year
MagPlex Beads	4°C in dark	6 months – 1 year
Antibody pairs as required for assay	Varies, most likely either +4 or -20°C	Varies
EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit (Pierce Cat #21935)	Varies by component	Varies
Biotin Quantitation Kit (Pierce Cat #28005)	Varies by component	Varies
Antibody Coupling Kit (Luminex Cat #B29045)	4°C	6 months – 1 year. One component is single use.
Streptavidin, R-phycoerythin (Invitrogen Cat #S866)	4°C in dark	6 months – 1 year

Are there any reagents that require additional preparation by the end-user?	See section above. System is open architecture and requires end-user to develop assays including labeling detector antibodies with biotin and coupling capture antibodies to MagPlex beads.
Are there any reagents that were not supplied by the company?	The antibody coupling kit is available separately from Luminex and is not included with device. All other reagents listed above are available from other sources. Luminex supplies a list of select vendors.

GENERAL INFORMATION	
Instrument Name	Cartridge Reader
Instrument Serial Number	0.0.4.2004
Firmware Version	A: 410091015113 B: 410091015114
Is this a COTS Instrument?	No
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	~ 3.5
System Footprint (w" x d" x h")	7 x 13.5 x 8
Total footprint with ancillary equipment (w" x d" x h")	No ancillary equipment required after firmware upgrade. Prior to upgrade, a laptop was required to analyze raw data.
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Instruction manual included
Was additional equipment required to power on the device? If so, explain.	Power cord supplied with device
Comments: None.	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	Refrigeration, wet ice
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	None after firmware upgrade, laptop before upgrade
Were there any special power requirements?	110V A/C, no battery
How much time was required to set up the instrument?	No set-up required other than plugging in power cord
How long did training take?	Simple to learn to operate. <10 minutes
Did you call the company with questions prior to starting your first run?	Yes. Inquired whether data existed for levels of detection
Did the company have a dedicated tech support person that was able to help you?	No. Worked mainly with CEO of company
Are any initial calibrations required? If so, insert a description and time required in Comments.	Yes
Comments: Training initially took longer because user was required to understand how to transfer data to an SD card and import it into Excel for analysis using a customized spreadsheet from MSD. Upon request, MSD installed an updated firmware that removes the requirement of exporting the raw data to excel for analysis. For initial calibration, lot parameters had to be downloaded to the device from a memory card supplied with the assays.	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
ECBC Assay Panel Cartridge	4°C	~ 1 year
Are there any reagents that require additional preparation by end-user?	No	
Are there any reagents that were not supplied by the company?	No	

GENERAL INFORMATION	
Instrument Name	RAPTOR
Instrument Serial Number	SF10047
Firmware Version	1.39
Is this a COTS Instrument?	Yes
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	12.3 w/o battery, 2.5 w/battery
System Footprint (w" x d" x h")	7.3 x 6.8 x 10.8
Total footprint with ancillary equipment (w" x d" x h")	N/A
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Yes
Was additional equipment required to power on the device? If so, explain.	No. Device was shipped with battery pack and A/C adapter.
Comments: Shipped incorrect A/C adapter. Received correct adapter to power device. Research International indicated looseness of power input (female end on device) was a design feature.	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	Reagents require storage at 4°C.
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	None. Computer is optional.
Were there any special power requirements?	Requires battery pack or A/C.
How much time was required to set up the instrument?	5 minutes
How long did training take?	About 240 minutes to read manual and understand system function.
Did you call the company with questions prior to starting your first run?	Yes. Contacted Dr. David McCrae.
Did the company have a dedicated tech support person that was able to help you?	No. Talked directly with Vice President.
Are any initial calibrations required? If so, insert a description and time required in Comments.	Device conducts a system check at power up. Takes seconds.
Comments: Waste bag vent port is plumbed to the sample reservoir which could cause contamination/defiling of sample. Manual is vague and contains typographical errors. Battery pack is non-rechargeable although a rechargeable option is available, battery discharged after less than two days of testing. Color coding/labeling of device inconsistent and incorrect. System contains a reagent cooler which needs to be frozen at -20°C prior to use. Maintains reagents at temperature ≤29°C for 24 hours.	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
RAPTOR Bioassay Coupon Kit	4°C	Production qualified for 3 to 6 months at 20°C.
Wash Buffer	4°C	Assigned 1 year expiration.
Are there any reagents that require additional preparation by end-user?	Yes. Wash buffer is 8.3mM Phosphate Buffer, pH 7.2 with 0.05% Triton X-100.	
Are there any reagents that were not supplied by the company?	Yes. Wash buffer. Vice President recommended ordering pre-weighed packets from Sigma.	

GENERAL INFORMATION	
Instrument Name	SpinDx
Instrument Serial Number	Unknown
Firmware Version	Unknown
Is this a COTS Instrument?	Yes
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	3.5
System Footprint (w" x d" x h")	6 x 6 x 6
Total footprint with ancillary equipment (w" x d" x h")	Dependent on laptop used. Communication with laptop is wireless via Bluetooth; therefore, laptop does not have to be located adjacent to device.
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No. System shipped in a pelican style case.
Were all the parts included in the shipment?	Yes
Was the training/operator manual included?	Yes. Shipment included software and training video. Training did not cover data analysis.
Was additional equipment required to power on the device? If so, explain.	System is powered by a built-in rechargeable battery pack.
Comments: None	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	Discs and lyophilized reagents require storage at 4°C.
Were the reagents received as required by the manufacturer	Yes. They were received on ice packs.
SET UP OF EQUIPMENT	
List ancillary equipment required	None
Were there any special power requirements?	No
How much time was required to set up the instrument?	Installation of Bluetooth adapter, virtual serial connection, and device OS software took about 30 minutes.
How long did training take?	10 minutes
Did you call the company with questions prior to starting your first run?	Yes. Asked them how to interpret the data. No information on data interpretation was sent with shipment.
Did the company have a dedicated tech support person that was able to help you?	No. Spoke directly to assay developer.
Are any initial calibrations required? If so, insert a description and time required in Comments.	Not initially.
Comments: This system requires pipetting small volumes of microbeads that may get trapped in a 10uL micropipette tip. A single volume micropipette and appropriate larger bore tips were shipped with the unit. Unit works via a Bluetooth enabled virtual serial port. After running initial assays, called the developer with concerns about system performance. At this time, they shipped calibrator beads to us. Calibrator beads indicated there is/are performance issues with instrument and data is unreliable. Will not assess detection of targets.	

REQUIRED REAGENTS, STORAGE, AND SHELF LIFE		
Reagent	**Storage**	**Shelf Life**
Disc	4°C	"A couple of months."
Lyophilized reagents for each assay	4°C	"A couple of months."
Are there any reagents that require additional preparation by end-user?	No	
Are there any reagents that were not supplied by the company?	No	

GENERAL INFORMATION	
Instrument Name	SPIRIT
Instrument Serial Number	Instrument does not have serial number
Firmware Version	Unknown
Is this a COTS Instrument?	Yes
ACTUAL EQUIPMENT SPECS	
Weight (lbs)	3
System Footprint (w" x d" x h")	11 x 7 x 7.5
Total footprint with ancillary equipment (w" x d" x h")	N/A
SHIPMENT OF EQUIPMENT	
Was there movement during shipment?	No
Were all the parts included in the shipment?	Multiple versions sent via email.
Was the training/operator manual included?	Received via email.
Was additional equipment required to power on the device? If so, explain.	Yes. Unit shipped with an external, rechargeable battery and A/C power adapter.
Comments: None	
SHIPMENT OF REAGENTS	
Did reagents require any special shipping?	Reagents shipped cold. Subsequent shipments were received without refrigeration and box did not indicate contents required refrigeration upon receipt.
Were the reagents received as required by the manufacturer?	Yes
SET UP OF EQUIPMENT	
List ancillary equipment required	Laptop computer, Serial-USB adapter, blunt end syringes
Were there any special power requirements?	No
How much time was required to set up the instrument?	See Comments.
How long did training take?	Two hours once on site.
Did you call the company with questions prior to starting your first run?	Yes
Did the company have a dedicated tech support person that was able to help you?	Yes
Are any initial calibrations required? If so, insert a description and time required in Comments.	Yes

Comments: Set-up involved opening a pelican case, installing two buffers and a waste tube, pre-wetting of the sensors, installation of specific and reference sensors, priming of the system, and referencing the sensors to a sucrose solution. Normally this process should take no more 30 minutes, but this system was fraught with problems.

The first system arrived without a functional peristaltic pump. This system was replaced with a device that appeared to work but did not record data from the *Y. pestis* chip (bad chip/bad software/unknown problem). The following day, the system became entirely unresponsive to user input. The company offered phone technical support and sent various versions of the instrument operating software to try to remedy the issues. The company finally replaced this device with the refurbished initial unit. The initial device again arrived without a functional peristaltic pump. At this point, we requested that Seattle Sensors travel to ECBC with a functional device. They set up and tested the device in our labs with our laptop and gave a training/introduction to the system. This third device appears to function appropriately.

Initial calibrations: Sensor chips must be calibrated against a high refractive index solution (sucrose) before first use. This involves injecting a 20-30% sucrose solution into the sample loop and allowing it to cover sensor surfaces. The computer software then has an algorithm to reference the sensors to the initiation solution. Additionally, running buffer must be passed across the sensors for enough time prior to sample analysis to allow a steady baseline to develop. The time required for this is subject to variations in the antibodies coated onto the sensor surfaces and is empirical.

Appendix D: Operational Assessment Table

Instrument: _____ Weather Conditions: _____
Time of Day: _____ Temperature: _____
Operator: _____ Humidity: _____

ATTRIBUTE	CATEGORY	DESCRIPTION	UNIT OF MEASURE	TEST SCORE	OPERATIONAL QUESTIONS
Set up Time for All Equipment	Usability	Time to set up all equipment (including ancillary equipment) for use	**Seconds**		
Programming Time	Usability	Time to program the instrument with information about sample to be analyzed	**Seconds**		
Training Time Required	Training	Time to become trained on the system	**Minutes**		
Sample Preparation Time in PPE (MOPP IV)	Sample Processing	Time required from receipt of sample until it is ready to be analyzed. Operator in PPE.	**Minutes**		
Sample Run Errors	Usability	Error during sample analysis	**Yes/No**		
Total Analysis Time	Sample Analysis	The duration of time to provide final identification of target from raw data.	**Minutes**		

Diagnostic Call (Identification/Positive/Negative/Unsure)_____

ATTRIBUTE	CATEGORY	DESCRIPTION	UNIT OF MEASURE	TEST SCORE				
				LOWEST	POOR	FAIR	GOOD	EXCELLENT
Ease-of-Use	Usability	User's Rating of ease to manipulate buttons, process samples, etc.	**Rating**					
Ease-of-Result Viewing and Interpretation of Data	Usability	User's Rating	**Rating**					
Supporting Documentation (e.g., technical manuals)	Usability	User's Rating	**Rating**					
Training Simplicity	Training	User's Rating	**Rating**					
Safety	Safety	User's Rating of safety	**Rating**					
Cleaning/Maintenance Simplicity	Logistical Support	User Rating	**Rating**					
Additional Comments								


EDGEWOOD CHEMICAL BIOLOGICAL CENTER
A U.S. Army RDECOM Laboratory