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Biodetection Technologies for First Responders: 2015 Edition

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May 2015

http://biodetectionresource.pnnl.gov

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Science and Technology

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Summary

Responding to a potential biological incident requires a number of competencies, including analyzing the incident, identifying methods of dissemination, identifying biological threat agents, planning the response, implementing the planned response, evaluating progress, and terminating the incident. The National Fire Protection Association (NFPA®) outlines the minimum required competencies in NFPA® 472.[1] Detailed standardized response protocols are given in ASTM E2770-10.[2]

When investigating a suspicious powder incident, a wide variety of sample collection products, field-deployable assays and detection systems can be used to determine if the substance contains biological material and warrants further investigation. First responders have several significant factors to consider before purchasing biological sampling and detection technologies, including the following:

- type of information obtained, usefulness and accuracy of results (performance)
- ease-of-use in the field
- total cost of ownership (e.g., hardware, consumables, and training needs)—understanding that reagent cost, shelf-life, instrument maintenance, and upgrades are significant contributors
- total time from sample to answer
- weight and size.

This guide summarizes a number of commercially available technologies that can be used by first responders in the field for the collection, screening, and identification of biological materials. *It is not meant to be an exhaustive list, nor an endorsement of any technology described herein*. Rather, this guide is meant to provide useful information about available technologies to help end-users make informed decisions about biodetection technology procurement and use. The summaries in this guide are based primarily on vendor-provided information; however, where possible the summaries have been supplemented with additional information obtained from publications, reports, and websites. Manufacturers were contacted and given the opportunity to verify the accuracy of technical specifications, available peer-reviewed references, and pricing. However, all information is subject to change from the time it was collected.

Comparing biodetection technologies is challenging in the absence of independent, standardized, third-party testing. Many factors can impact measured performance metrics, such as sensitivity (limit of detection), selectivity (cross-reactivity), and reliability (the occurrence of false-positive or false-negative results). Environmental conditions, sample type, biothreat agent, and degree of sample preparation all impact a technology's performance and make it difficult to directly compare data generated for different technologies tested under different (and often not well-defined) conditions. Vendor-provided performance metrics are listed, and where possible, shown in relation to the quantity or concentration of organism detected. When available, peer-reviewed publications that evaluate the performance of a technology have been cited; however, such publications are rare and often outdated due to ongoing technology improvements by vendors. Available peer-reviewed references are listed along with a short summary of

[1] Annex B: Competencies for Operations Level Responders Assigned Biological Agent–Specific Tasks. In *Standard for Competence of Responders to Hazardous Materials/Weapons of Mass Destruction Incidents;* NFPA 472; National Fire Protection Association: Quincy, MA, 2013; pp. 86-91.

[2] *Standard Guide for Operational Guidelines for Initial Response to a Suspected Biothreat Agent*; ASTM E2770-10; American Society for Testing and Materials, Subcommittee E54.01: West Conshohocken, PA, 2010. DOI: 10.1520/E2770-10.

findings in each paper. Publically available peer-reviewed references include a hyperlink. A digital object identifier (DOI) number is given for most publications to assist finding the specific article online, however access to the entire electronic publication will depend on the user's or organization's access rights.

The quality of a company's management system can also impact product quality; therefore we provide information about some International Organization for Standardization (ISO) certifications. While having a certified management system helps to validate that certain requirements are being met, it is not a prerequisite for producing an effective and high-quality product. In this guide, we note whether the company is ISO 9001:2008-certified (i.e., specifies the requirements of a quality management system) or ISO 13485:2003-certified (i.e., specifies the requirements of a quality management system for medical devices). The companies included in this guide may hold additional ISO certifications (e.g., for an environmental management system or an occupational health and safety management system); however, those certifications are not listed here.

Other information that may aid in the evaluation of a products's effectiveness are designations given by the U.S. Department of Homeland Security (DHS) as part of its Support Anti-terrorism by Fostering Effective Technologies (SAFETY) Act of 2002 (www.safetyact.gov). The SAFETY Act, enacted as part of the Homeland Security Act of 2002, facilitates the development and deployment of effective anti-terrorism technologies by creating risk- and litigation-management systems. Companies can submit applications to DHS for review of their technology or services. Products can achieve one of three levels of DHS-designated effectiveness:

1. Developmental Testing and Evaluation Designation (DTED) (needs more proof, but potential exists)
2. Designated (proven effectiveness, with confidence of repeatability)
3. Certified (consistently proven effectiveness, with high confidence of enduring effectiveness).

Products having any one or more of these designations or certifications (DTED, Designated, and/or Certified) are listed on the SAFETY Act website on an "Approved Technologies" tab.[3] Where applicable, SAFETY Act designations and certifications are noted in this guide, though the lack of a designation or certification does not signify that a product is not effective.

The focus of this guide is on available products for environmental sampling and detection and not products for clinical samples, food, or other sample types. The products are presented in four groups as follows:

- sample collection kits and tools
- general biological indicator tests including protein, adenosine triphosphate (ATP), deoxyribonucleic acid (DNA)/ribonucleic acid (RNA), and spectroscopic (Fourier Transform Infrared [FTIR]) technologies
- immunoassays
- polymerase chain reaction-based (PCR) detection systems.

Table ES.1 through Table ES.4 provide an overview of the technologies described in this guide, including the product name, manufacturer, manufacturer website, cost, and applicable notes.

[3] SAFETY Act website – https://www.safetyact.gov

Table ES.3. Immunoassay-Based and Miscellaneous Detection Products for Potential Biothreats

Product Name	Manufacturer	Manufacturer Website	Cost	Notes
BADD™	AdVnt Biotechnologies, LLC	http://www.advnt.org	$26/agent	1-agent assays. 10 assays per box.
Pro Strips™	AdVnt Biotechnologies, LLC	http://www.advnt.org	$73/assay $15/agent	5-agent assays. 10 assays per box.
BioDetect™ Test Strips with optional Guardian or Defender reader	Alexeter Technologies, LLC	http://www.alexeter.com	$27/agent	1-agent assays. 25 assays per box. Optional optical readers: Guardian ($7500) or Defender ($9995).
RAID™ Multi-Test Strips	Alexeter Technologies, LLC	http://www.alexeter.com	$50-$100/assay $12-$17/agent	5- or 8-agent pathogen assays, 3-agent toxin assay. 10 assays per box.
NIDS® assays and optical reader	ANP Technologies®, Inc.	http://anptinc.com/	$60-$80/assay $20/agent	3 and 4-agent assays. Assays sold individually. Optional optical reader ($6900).
IMASS assays	BBI Detection, LLC.	http://www.bbidetection.com	$127/assay $16/agent	8-agent assay with integral sampling sponge and buffer. 10 assays per box.
Portable Toxin Detector (pTD)	Bruker Daltronics	http://www.bruker.com	$126/assay $25/agent	5-agent assays. 15 assays per box. Instrument cost $69,000.
ENVI Assay System and optional reader	Environics, Inc.	http://www.environicsusa.com	$40-$65/agent	1-agent assays. 10 assays per box. Optional optical reader and PC software, PC not included ($4500) or ChemPro®100 module ($15,000).
Toxin Screen	GenPrime, Inc.	http://www.genprime.com	$100/assay $33/agent	3-agent assay. Assays sold individually.
MENTOR 100-Biodetector	Menon Biosensors, Inc.	http://www.menon.us	$8-$20/assay	Immunoassay- and nucleic acid-based probe Nuclear Magnetic Resonance (NMR) biodetector ($25,000).
Lab-in-the-Box MENTOR Biodetector	Menon Biosensors, Inc.	http://www.menon.us	$8-$20/assay	Lab-in-the-Box immunoassay- and nucleic acid-based probe Nuclear Magnetic Resonance (NMR) biodetector ($15,000).
PR2 1800	Meso Scale Defense™	http://www.mesoscaledefense.com	$1-$4/assay	Multiplexed electrochemiluminescent immunoassay system in 96-well plate format ($80,000).
KDTB Gold®	NBC-SYS	http://www.nexter-group.fr/nexter/Flipping_Book/Export_FR/#198	$54/assay	1-agent assays. 5 assays each for 8 different biothreats included in one kit ($2150). Optional optical reader ($3225).
Smart™ II		http://www.nhdiag.com	$23/agent	1-agent assays. 25 assays per box.
CANARY® Zephyr	PathSensors, Inc.	http:www.pathsensors.com	$16/agent	Automated 1-agent cell-based assays (5 assays per container) and detection instrument ($23,500).

Acronyms and Abbreviations

ABICAP	AntiBody Immuno Column for Analytical Purpose
ASTM	American Society for Testing and Materials
ATP	adenosine triphosphate
ATR	attenuated total reflection
BHI	brain heart infusion
CBRE	chemical, biological, radiological, and explosive
CDC	Center for Disease Control
CFU	colony-forming units (equivalent to number of organisms)
DHS	U.S. Department of Homeland Security
DOI	digital object identifier
DNA	deoxyribonucleic acid
DTED	Developmental Testing and Evaluation Designation
ELISA	enzyme-linked immunosorbent assay
FAC	Forensic Analytical Center
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
GE	genome equivalent
GPS	global positioning system
HazMat	hazardous materials
ILV	independent laboratory validation
ISO	International Organization for Standardization
LFA	lateral flow assay
LFD	lateral flow device
LOD	limit of detection
LRN	Laboratory Response Network
MD	(AOAC) Method Developer
NFPA®	National Fire Protection Association
NMR	Nuclear Magnetic Resonance
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDA	personal digital assistant
PFU	plaque-forming units (equivalent to number of viruses)
pg	picrogram (one trillionth of a gram)
PNNL	Pacific Northwest National Laboratory
RF	radio frequency
RLU	relative light units
RNA	ribonucleic acid
SAFETY Act	The Support Anti-terrorism by Fostering Effective Technologies Act
SAS	Small Area Sampling
SEB	Staphylococcal Enterotoxin type B
SPADA	Stakeholder Panel on Agent Detection Assays

TICS	toxic industrial chemicals
TIMS	toxic industrial materials
TIRF	total internal reflectance fluorescence
WMD	weapon of mass destruction

1.0 Biothreat Diseases and Causative Agents

Important Note: Biothreat agent names used throughout this guide are those specified by equipment manufacturers. Often, vendors reference a disease instead of its causative agent. One prevalent example is the use of anthrax, a disease caused by the organism *Bacillus anthracis.* These tests detect the organism, not the disease, yet anthrax and *Bacillus anthracis* are often used interchangeably in the biodetection technology marketplace, as are the terms plague and *Yersinia pestis*.

Table 1.1 and Table 1.2 list diseases/toxins and their causative agents/sources. These tables are separated into three categories according to the priority pathogen lists at the Center for Disease Control (CDC) website.[1] The CDC website also includes a wealth of information on bioterrorism and different biothreats, including basic descriptions of biothreat agents, risk factors, symptoms, and medical care. The priority pathogen lists are periodically reviewed and revised in conjunction with the U.S. Department of Homeland Security (DHS) and the CDC. First responders and the public health system must be prepared to address various biological agents, even those that are uncommon in the United States.

High-priority pathogens (i.e., Category A) are those organisms or biological agents that pose a risk to national security because they:

- are easily disseminated or transmitted between people
- have potential for high mortality rates
- have potential for major public health impacts including public panic and social disruption
- require special actions for public health preparedness.

Table 1.1. Diseases/Toxins for Category A Priority Pathogens

Disease/Toxin	Causative Agent/Source
Anthrax	*Bacillus anthracis*
Botulism	*Clostridium botulinum* toxin
Plague	*Yersinia pestis*
Smallpox	Variola (or orthopox) virus
Tularemia	*Francisella tularensis*
Viral hemorrhagic fevers	Arenaviruses (e.g., Lassa and Machupo)
	Filoviruses (e.g., Ebola and Marburg)

Category B pathogens are the second highest priority organisms/biological agents and are those that have moderate ease of dissemination, moderate morbidity rates and low mortality rates, and require specific enhancements of the CDC's diagnostic capacity and disease surveillance.

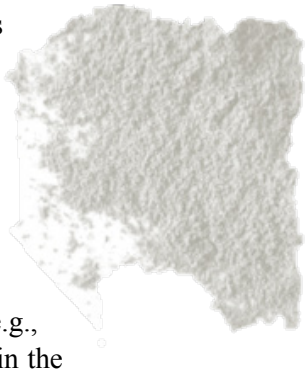
[1] CDC website – http://www.bt.cdc.gov/agent/agentlist-category.asp

Table 1.2. Diseases/Toxins for Category B Priority Pathogens

Disease/Toxin	Causative Agent/Source
Brucellosis	*Brucella* species
Epsilon toxin	*Clostridium perfringens*
Food safety threats	(e.g., *Salmonella* species, *Escherichia coli* (*E. coli)* O157:H7, *Shigella*)
Glanders	*Burkholderia mallei*
Melioidosis	*Burkholderia pseudomallei*
Psittacosis	*Chlamydia psittaci*
Q fever	*Coxiella burnetii*
Ricin toxin	*Ricinus communis*
Staphylococcal enterotoxin B (SEB)	*Staphylococcus aureus*
Typhus fever	*Rickettsia prowazekii*
Viral encephalitis	Alphaviruses (e.g., Venezuelan equine encephalitis, eastern equine encephalitis, and western equine encephalitis)
Water safety threats	(e.g., *Vibrio cholera* and *Cryptosporidium parvum)*

The third highest priority organisms/biological agents (i.e., Category C) include emerging pathogens (e.g., Nipah virus and hantavirus) that could be engineered for mass dissemination in the future due to their ease of availability, ease of production and dissemination, and potential for high morbidity and mortality rates and health impact.

2.0 Sample Collection



While some biodetection systems for analyzing suspicious powders provide tools for sampling, many do not. Sampling kits are available in a wide range of configurations. Typically, these kits consist of a swab or scoop to pick up the sample and a collection vial with buffer (often phosphate buffered saline [PBS]) to solubilize or suspend the sample. Additional features may include droppers for sample dispensing, chain-of-custody forms, or sample preparation reagents for removal of potential assay inhibitors. Some sample containers or outer packaging bags can be sealed and are designed to be dunked into a decontamination solution (e.g., bleach), so that the sample can be sent to a centralized laboratory or tested in the warm zone (outside of the hot [i.e., contaminated] zone). Standardized practices for the collection of visible powders suspected of being biothreat agents have been developed by the American Society for Testing and Materials (ASTM) (1).

This guide includes information pertaining to sample collection kits for sampling solid powders or material from surfaces—some also work with liquid samples. Aerosol samplers and dedicated liquid samplers are not included in this report. Most kits are designed to suspend suspect material in a buffered solution for downstream analysis. With a few exceptions, the majority of the kits provide no sample processing to remove potential assay inhibitors. Most kits are designed to suspend suspect material in a buffered solution for downstream analysis. Although most of the kits themselves have not been formally evaluated, many of the primary components of the kits (e.g., swabs, wipes, and sponges) have been evaluated for their ability to recover *Bacillus* species spores from various surfaces (2-3). A large number of sample collection studies have been conducted and only a few examples are given in this guide.

Most available literature on sampling materials concerns recovery efficiency. Recovery efficiency is affected by a number of factors including the sampling materials (e.g., cotton, foam, or polyester), surface area covered, type of surface (e.g., stainless steel, tile, carpet, or drywall), the assay used to quantify recovery, and even the spore deposition method (2-3). The range of results in these published studies suggests that the best approach for sampling suspicious powders during suspected incidents will depend on factors such as the amount of material available, the sampling material, the type of sampling surface, and the downstream detection method(s).

When choosing a sampling kit, care should be exercised to ensure that, especially when buffers are used, the final solubilized or suspended sample is compatible with the downstream detection methods. For example, some sample buffers have components that can interfere with immunoassays or polymerase chain reaction (PCR)-based detection systems. Always verify with the sampling kit and detection technology manufacturers that the sampling kit buffer is compatible with the chosen detection approach.

References

1. *ASTM. Standard Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biothreat Agents from Nonporous Surfaces*; ASTM E2458-10; American Society for Testing and Materials, Subcommittee E54.01: West Conshohocken, PA, **2010**. DOI: 10.1520/E2458-10.

This standard provides detailed step-by-step guidance for collection of bulk (Method A) and residual (Method B) suspicious powders after a sample has been screened for explosive, radiological, and acute chemical hazards. These sampling practices are performed as part of a risk assessment (i.e., hazard assessment and threat evaluation) in coordination with the Federal Bureau of Investigation as described in ASTM E2770-10. The bulk sample collected by Method A is intended to be packaged and transported to a Laboratory Response Network (LRN) reference lab. Swab sampling of residual powder (Method B) can be used for onsite biological screening. This standard provides a detailed list of sampling and packaging equipment and supplies for each method. Both methods use a two-person team (sampler and assistant sampler). Multiple example forms are provided as part of the standard and include: a field-screening results form, a sample collection sheet, a chain-of-custody form, and example biothreat tracking and specimen submission forms from the New York State Department of Health and Massachusetts Department of Public Health.

2. Rose, L. J., L. Hodges, H. O. O'Connell, and J. Nobel-Wang. National Validation Study of a Cellulose Sponge Wipe-Processing Method for Use After Sampling *Bacillus anthracis* Spores from Surfaces. *Appl. Environ. Microbio*l. **2011**, *77*, 8355-8359. DOI: 10.1128/AEM.05377-11.

   Nine LRN laboratories evaluated 3M cellulose sponges (pre-moistened) for sampling *Bacillus anthracis* Sterne strain spores from 10-in. square steel surfaces. Spores, dust, and background organisms were applied to the surfaces at levels ranging from 10-10,000 spores. Approximately seven sponges and two positive control wipes were tested at each site. Percent recovery ranged from 24 to 32% with coefficients of variation (% CV) of 20 of 31% for between-lab and 20 to 69% for within-lab samples. The presence of dust and background organisms did not appreciably impact the ability to detect *Bacillus anthracis.* The low levels of spores used in this study highlighted the large variability inherent in the sampling process.

3. Edmonds, J. M., P. J. Collett, E. R. Valdes, E. W. Skowronski, G. J. Pellar, and P. A. Emanuel. Surface Sampling of Spores in Dry-Deposition Aerosols. *Appl. Environ. Microbiol*. **2009**, *75*, 39-44. DOI: 10.1128/AEM.01563-08.

   This study compared recovery of spores deposited onto surfaces in dry (aerosol) and liquid form (spores were applied to a surface in a suspension and the surface was allowed to dry). Four different 2-cm x 5-cm surfaces were used (i.e., glass, painted steel, polycarbonate, and vinyl tile). Four different swab materials (3 to 4 replicates each) were tested: Fisher Scientific Puritan cotton swabs, Fisher Scientific FisherBrand Dacron-tipped swabs, Starplex Scientific rayon-tipped swabs, and scientific supplier VWR Critical Swab polyurethane macrofoam-tipped swab. Percent recoveries and reproducibility (% CV) were impacted by the surface material, the swab type, and the spore deposition method. CVs ranged from 10 to 35% across all variables studied and were consistently nearly twice as high for liquid than for aerosol-deposited spores on vinyl tile. All swab types performed well for collection from glass surfaces after liquid deposition (82 to 89% recovery), but percent recoveries were lower for aerosol deposition (62 to 65%). Spore recovery from painted steel surfaces was generally the lowest (42 to 58%) for all swab types and deposition methods. Different swabs gave higher recoveries depending on the sample surface and spore deposition method. The macrofoam swab performed better in most, but not all, instances. In a separate experiment, the recovery of liquid-deposited spores from glass surfaces was shown to be dependent on the number of spores present on the surface. For example, four applied spore levels (i.e., 10^4, 10^5, 10^6, and 10^7) had recoveries of 42, 61, 76, and 93%, respectively.

Menon Biosensors, Inc.: MENTOR-100 Biodetector

Phone: (858) 675-9990 *Manufacturer's website*: http://menon.us/

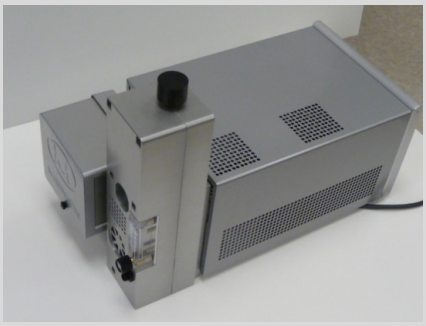
Technology Summary

The MENTOR-100 is based on Molecular Mirroring (M_2) NMR platform technology that can be configured for both nucleic acid assays and immunoassays using the same platform. The MENTOR 100 Biodetector collects particles and passes them through a microfluidic system where pathogen targets become bound to immobilized probes on the nanoparticles. These bound markers are detected and identified by magnetic resonance technology. The system can detect pathogens or toxins from aerosol, hydrosol, soil, and powder matrices. The system performs aerosol collection, concentration, nucleic acid, or immunoassay signal amplification and detection, followed by automatic decontamination of the fluidic system.

For nucleic acids, the M^2 Nuclear Magnetic Resonance (NMR) bioassay uses oligonucleotide probes conjugated to nanoparticles that target pathogen DNA sequences. The very long spin relaxation time of water protons allows the measurement of the NMR spin-spin relaxation time (T_2) to detect as few as 10 CFU in a liter of air. With no target present, the nanoparticles are uniformly distributed in the assay and the relaxation time (T_2) is short. However, when a target DNA is present the oligonucleotide/streptavidin-coated nanoparticles react differently when bound to the target DNA. Thus, the measured value of T_2 determines the presence or absence of the target DNA. Detection is determined by changes in the reported T_2 measurement values.

The autonomous MENTOR-100 consists of the following subsystems: An aerosol collector that can capture and concentrate both viruses and bacteria and a sampling system configured to process swab, hydrosol, powder, and soil samples with minimal sample preparation using supplied reagents.

Specifications



MENTOR-100 Immunoassay- and Nucleic Acid-Based Probe NMR Biodetector

Biothreat Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD**
Anthrax	*Bacillus anthracis*	Not reported
Plague	*Yersinia pestis*	Not reported
Tularemia	*Francisella tularensis*	Not reported
Toxins	Various	Not reported

Assay time: *45-60 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes.*
Unit weight: *20 lb.*
Power: *AC.*
Cost: *Assay – $8-$20 (volume and target dependent); instrument – $25,000.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *12 months from date of manufacture.*

Peer-Reviewed References

No peer-reviewed publications were found that evaluate the use of this product for the detection of biothreat agents.

Menon Biosensors, Inc.: Lab-in-the-Box MENTOR Biodetectors

Phone: (858) 675-9990 *Manufacturer's website*: http://menon.us/

Technology Summary

The Lab-in-the-Box MENTOR biodetectors are portable systems primarily marketed to first responders for use in the field. From 24 to 96 samples can be processed by a user at a time.

The system can be configured to perform both nucleic acid assays as well as immunoassays. For nucleic acids, amplification is performed using a thermal cycler provided with the system. For immunoassays, signal amplification methods are used to increase the sensitivity.

Custom-designed oligonucleotide probes or antibodies conjugated to magnetic nanoparticles are used to bind to specific pathogen targets (nucleic acids and toxins) resulting in an increased spin relaxation time compared to unbound probes or antibodies. For immunoassays, proprietary signal amplification methods are used.

The Lab-in-the-Box system and accessories for performing the assay are packaged inside a commercial enclosure (~20 in. x 15 in. x 9 in.). Samples can be processed using fixed volume pipettes. Up to 96 samples can be measured within 2 hours. The system can process swab, hydrosol, powder, and soil samples with minimal sample preparation. The Mini-MENTOR can process up to 4 samples in 45 minutes. The Mini-MENTOR system and a tablet computer are packaged in a case (~13 in. x 12 in. x 6 in.).

Specifications

Lab-in-the-Box MENTOR and Mini-MENTOR Immunoassay- and Nucleic Acid-Based Probe NMR Biodetectors

Biothreat Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD**
Anthrax	*Bacillus anthracis*	Not reported
Plague	*Yersinia pestis*	Not reported
Tularemia	*Francisella tularensis*	Not reported
Toxins	Various	Not reported

Assay time: *45-120 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes.*
Unit weight: *30 lb.*
Power: *AC and battery versions available.*
Cost: *Assay – $8-$20 (volume and target dependent); instrument – $15,000.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *12 months from date of manufacture .*

Peer-Reviewed References

No peer-reviewed publications were found that evaluate the use of this product for the detection of biothreat agents.