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Biodetection Technologies for First Responders

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November 2012



Pacific Northwest
NATIONAL LABORATORY

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Science and Technology

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PACIFIC NORTHWEST NATIONAL LABORATORY
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Pacific Northwest National Laboratory
Richland, Washington 99352

Summary

In a white powder scenario, a large variety of field-deployable assays can be used to determine if the suspicious substance contains biological material and warrants further investigation. First responders have several significant factors to consider before purchasing biodetection technologies, including:

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

This report summarizes commercially-available, hand-portable technologies that can be used by first responders in the field. This is not meant to be an exhaustive list, nor an endorsement of any technology described herein. Rather, this report is meant to provide useful information about available technologies to help end-users make informed decisions about biodetection technology procurement and use. Information listed in this report is primarily vendor-provided; however, where possible it has been supplemented with additional information obtained from publications, reports, and websites. Manufacturers were given the chance to review summaries of their technologies from August through November 2012 to verify the accuracy of technical specifications, available references, and pricing.

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) and selectivity (cross-reactivity). Environmental conditions, sample type, target threat agent, and degree of sample preparation all impact a technology's performance and make it difficult, if not impossible, to directly compare data generated for different technologies tested under different (and typically not well-defined) conditions. Vendor-provided performance metrics are listed, and where possible, shown in relation to the quantity (rather than concentration) of organism detected, because different technologies require different sample volumes (which ultimately affects the sensitivity of a test). Where possible, publicly available, peer-reviewed publications that evaluate the performance of a technology have been used; however, such publications are rare and often outdated. Available references are listed along with a short summary of findings and the technology's applicability for testing solid samples (e.g., powders).

This report has been organized based on grouping similar technologies relevant to investigating potential biological threat incidents. These include the following:

- sample collection kits
- non-specific assays including protein, adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), and spectroscopic assays
- immunoassays
- polymerase chain reaction (PCR)-based detection systems.

Table ES.1 provides an overview of the technologies described in this report, including the product, manufacturer, website, cost, and applicable notes.

Table ES.1. Overview of Products for Sampling and Detection of Potential Biothreats

Instrument/Kit	Manufacturer	Company Website	Type of Assay	Cost	Notes
Alexeter Collection Swab™	Alexeter	http://www.alexeter.com/biow/index.asp	Sampling Kit	\$75/box of 25	compatible with PCR and immunoassay
Sample Collection and Recovery Device (SCRD)	ASD BioSystems	http://www.asdbiosystems.com/p1.htm	Sampling Kit	\$12 each	compatible with PCR and immunoassay
SWIPE™ Kits	New Horizons Diagnostics	http://www.nhdiag.com/profile_one.shtml	Sampling Kit	Kit dependent \$13.20-23.10	compatible with PCR and immunoassay
B2C™ Bulk Sample Collection Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com/	Sampling Kit	\$26.71 each	meets American Society for Testing and Materials (ASTM) Standard E 2458-10 for bulk sample collection
Biological Sampling Kit (BiSKit™) - Large Area	QuickSilver Analytics, Inc.	http://www.chembiokits.com/	Sampling Kit	\$31.50 each	large area sampling kit compatible with PCR and immunoassay
QSA Model 102™ Full Forensic Analytical Center (FAC)	QuickSilver Analytics, Inc.	http://www.chembiokits.com/	Sampling Kit	\$2961.80 each	large area sampling kit developed and used by U.S. Army Mobile Labs and Kits Team
Mini Push Pack™ Kits Wipe/Bio/Solid/Liquid	QuickSilver Analytics, Inc.	http://www.chembiokits.com/	Sampling Kit	\$36.70 each	single-use kits
Small Area Sampling (SASTM) Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com/	Sampling Kit	\$17.96 each	designed for small area sampling; 4-year shelf-life
S3™ Bio Sampler	QuickSilver Analytics, Inc.	http://www.chembiokits.com/	Sampling Kit	\$22.56 each	Phosphate buffered saline (PBS) buffer-moistened sponge for sampling; compatible with PCR and immunoassay
LiNK2®	Triarii Scientific, LLC	http://www.tri-sci.com/Link2.html	Sampling kit	\$55 each	removes some PCR inhibitors; not compatible with immunoassay
HAZCAT WMD Kit	HazTech Systems	http://www.hazcat.com	Assorted	\$4, 910.17	an all-in-one chemical/biological/radiological/nuclear/explosive detection and classification kit
Prime Alert®	GenPrime	http://www.genprime.com/	Non-Specific Biological (DNA)	\$12,000 reader; \$200/test	non-specific DNA colorimetric test with a separate immunoassay toxin test

Table ES.1. (continued)

Instrument/Kit	Manufacturer	Company Website	Type of Assay	Cost	Notes
BioCheck® Powder Screening Kit	20/20 Bioresponse	http://biocheckinfo.com/	Non-Specific Biological (Protein & pH)	\$687.50/25 kits	non-specific colorimetric protein detection and pH test
Indipro	Macherey-Nagel	http://www.mn-net.com/tabid/10441/default.aspx	Non-Specific Biological (Protein)	\$103/50 strips	non-specific protein detection
Profile® 1	New Horizons Diagnostics	http://www.nhdiag.com/profile_one.shtml	Non-Specific Biological (ATP)	\$5000 reader: \$450/100 tests	non-specific test for live bacterial cells using ATP
HazMatID™ 360	Smiths Detection	http://www.smithsdetection.com	Non-Specific Biological (FT-IR/Protein)	\$53,000	non-specific test for biological material (protein-based) using FT-IR spectroscopy
HazMatID Ranger™	Smiths Detection	http://www.smithsdetection.com	Non-Specific Biological (FT-IR/Protein)	\$35,000	non-specific test for biological material (protein-based) using FT-IR spectroscopy
HazMatID™ Elite	Smiths Detection	http://www.smithsdetection.com	Non-Specific Biological (FT-IR/Protein)	\$50,000	non-specific test for biological material (protein-based) using FT-IR spectroscopy
TruDefender™ FT/FTi	Thermo Scientific (formerly Ahura Scientific)	http://www.thermoscientific.com	Non-Specific Biological (FT-IR/Protein)	\$45,000-\$46,500	non-specific test for biological material (protein-based) using FT-IR spectroscopy
BADD™ Biowarfare Agent Detection Devices	Advnt	http://www.advnt.org	Immunoassay	\$24.50/test	single-agent lateral flow immunoassay
Pro Strips™	Advnt	http://www.advnt.org	Immunoassay	\$699.50/10 tests	five-agent lateral flow immunoassay
BioDetect™ Test Strips	Alexeter	http://www.alexeter.com/biow/products.asp	Immunoassay	\$685/25 tests	single-agent lateral flow immunoassay
Defender TSR™ reader for use with BioDetect™ Test Strips	Alexeter	http://www.alexeter.com/biow/products.asp	Immunoassay reader	\$6500 reader	handheld optical reader for single-agent BioDetect™ test strips using Windows Mobile interface

Table ES.1. (continued)

Instrument/Kit	Manufacturer	Company Website	Type of Assay	Cost	Notes
Guardian Reader™ for use with BioDetect™ Test Strips	Alexeter	http://www.alexeter.com/biow/products.asp	Immunoassay reader	\$9995 reader	optical reader for single-agent BioDetect™ test strips
RAID™ Multi-Test Strips	Alexeter	http://www.alexeter.com/biow/products.asp	Immunoassay	\$695-\$995/10 tests	multiplexed lateral flow immunoassay available as either five-plex or eight-plex
ENVI Assay System	EnviroNics	http://www.environicsusa.com	Immunoassay	\$400-\$450/10 tests	toxin assays can be read visually or with a reader (\$3452), while the ENVI-FL pathogen assays require a reader
BIOSENSOR™ 2200R	MSA/QTL	http://www.msafire.com/	Immunoassay, automated	\$16,450 \$80/single-agent test	fluorescence-based automated immunoassay detection system
Smart™ II	New Horizons Diagnostics	http://www.nhdiag.com/profile_one.shtml	Immunoassay	\$550/25 tests	single-agent lateral flow immunoassay strips
RAMP®	Response Biomedical	http://www.responsebio.com	Immunoassay and reader	\$11,333 \$27/test (anthrax)	fluorescence-based lateral flow immunoassay strips and reader
RAPTOR™: Portable, Multianalyte Bioassay Detection System	Research International	http://resrchintl.com/	Immunoassay, automated	\$50,000 \$200/test	portable automated immunoassay system designed for the U.S. military
NIDS™	ANP Technologies/Smiths Detection	http://www.smithsdetection.com/NIDS.php	Immunoassay and reader	\$7500, \$45-\$75/assay	multiplexed lateral flow immunoassay with optical reader
BioThreat Alert® and Reader	Tetracore	http://www.tetracore.com/bio-warfare/	Immunoassay and reader	\$5500, \$605/box of 25	single-agent lateral flow immunoassay with optical reader
FilmArray®	BioFire Diagnostics (formerly Idaho Technology)	http://www.biofiredx.com	PCR	\$49,500, \$180 pouch	sample-to-answer PCR system, detects 21 targets per pouch
R.A.P.I.D.® (JBAIDS)	BioFire Diagnostics (formerly Idaho Technology)	http://www.biofiredx.com	PCR	\$55,000, \$452/ 48 assays	portable PCR system designed for the U.S. Military; multiple assays available

Table ES.1. (continued)

Instrument/Kit	Manufacturer	Company Website	Type of Assay	Cost	Notes
RAZOR® EX	BioFire Diagnostics (formerly Idaho Technology)	http://www.biofiredx.com	PCR	\$38,500, \$200 pouch	portable PCR system detects 10 targets per pouch
Bio-Seeq™ PLUS	Smiths Detection	http://www.smithsdetection.com	PCR	\$45,000, \$30/assay	portable PCR system, uses single- agent assays
T-COR 4™	Tetracore	http://www.tetracore.com/t-cor/index.html	PCR	\$16,000, \$12 cartridge	portable PCR system, uses single- agent assays

Acronyms and Abbreviations

ASTM	American Society for Testing and Materials
ATP	adenosine triphosphate
COTS	commercial-off-the-shelf
DNA	deoxyribonucleic acid
ECBC	Edgewood Chemical and Biological Center
EEE	Eastern Equine Encephalitis
EPA	U.S. Environmental Protection Agency
ETV	U.S. EPA Technology Verification
FAC	Forensic Analytical Center
FT-IR	Fourier Transform Infrared
HAZMAT	hazardous materials
LFA	lateral flow assay
LOD	limit of detection
NHD	New Horizons Diagnostics
NIDS	Nano Intelligent Detection System
NYDOH	New York State Department of Health
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PNNL	Pacific Northwest National Laboratory
R.A.P.I.D.	Ruggedized Advanced Pathogen Identification Device
RAMP	Rapid Analyte Measurement Platform
RNA	ribonucleic acid
SCRD	sample collection and recovery device
SEB	Staphylococcal Enterotoxin B (toxin)
SPADA	Stakeholder Panel on Agent Detection Assays
TNT	trinitrotoluene
UAB	University of Alabama
WEE	Western Equine Encephalitis
WMD	weapon of mass destruction

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1.0 Biothreat Diseases and Causative Agents

Important Note: Biothreat detection target names used throughout this report 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* (*B. anthracis*). These tests detect the organism, not the disease, yet anthrax and *B. anthracis* are used interchangeably in the biodetection technology marketplace. Table 1.1 lists diseases and their corresponding biothreat agents.

Table 1.1. Causative Agents or Sources for Various Diseases and Toxins

Disease/Toxin	Causative Agent/Source
Anthrax	<i>Bacillus anthracis</i>
Plague	<i>Yersinia pestis</i>
Tularemia	<i>Francisella tularensis</i>
Smallpox	Orthopoxvirus (Variola virus)
Brucellosis	<i>Brucella species</i>
Typhus	<i>Rickettsia prowazekii</i>
Viral encephalitis	Alphaviruses (Western Equine Encephalitis [WEE], Venezuelan Equine Encephalitis [VEE], and Eastern Equine Encephalitis [EEE])
Viral hemorrhagic fevers	Marburg and Ebola Viruses
Q fever	<i>Coxiella burnetii</i>
Melioidosis	<i>Burkholderia pseudomallei</i>
Glanders	<i>Burkholderia mallei</i>
Foodborne illness	<i>Salmonella species</i> , <i>E. coli</i> O157:H7, <i>Shigella</i>
Ricin toxin	<i>Ricinus communis</i> (Castor oil plant)
Staphylococcal Enterotoxin B (SEB) toxin	<i>Staphylococcus aureus</i>
Botulism (botulinum toxin)	<i>Clostridium botulinum</i>

2.0 Sample Collection

In addition to sampling kits provided as part of an assay kit, wide ranges of standalone sampling kits are available. Typically, these kits consist of a swab or scoop to pick up the sample and a collection vial with buffer to solubilize the sample. Additional features may include integrated droppers for sample dispensing, chain-of-custody forms, or sample preparation for removal of potential assay inhibitors. Most kits are designed to be dunked for decontamination, so that a sample can easily be sent out to a laboratory or tested in the warm or cold zone (near or outside of the contaminated area, respectively).



For the most part, the sampling kits listed in this report are designed to sample “white powders.” With a few exceptions, no sample processing to remove potential assay inhibitors is employed. The kits are designed to suspend suspect material in a buffered solution for downstream analysis. Whereas the kits themselves have not been formally evaluated, principal components of the kits (e.g. swabs, wipes, and sponges) have been evaluated for their ability to recover *Bacillus spp.* spores from various surfaces (1-6). In some cases, the protocols and components provided in the kits are designed to meet standards for law enforcement/chain of custody requirements for the criminal investigation of a biological attack (7-9).

Most of the available literature on sampling materials concerns recovery efficiency. Recovery efficiency is affected by a number of factors including the sampling materials, surface area covered (1), inanimate surfaces (stainless steel, tile, carpet, drywall, etc.), how assayed, and even spore deposition (1-6). With these studies, it is important to emphasize that there is an underlying assumption that high spore/agent concentrations will be present in a small area. Unfortunately, due to the multifactorial nature of the sampling and preservation issues, the studies offer little consensus on best practices for sampling “white powders” from suspected incidents.

In summary, when choosing a sampling kit, the potential downstream diagnostic assays should be considered. Care should be exercised to insure that, especially when buffers are used, the final sample is compatible with the down-stream detection methods. For example, some sample buffers have components that can interfere with immunoassays or PCR-based detection systems. Always verify with the manufacturer that the sampling kit is compatible with the diagnostic assay and suitable for collection of the sample type of interest.

References

1. **Buttner, M. P., P. Cruz, V. L. Stevens, and P. A. Emanuel.** 2004. Evaluation of the Biological Sampling Kit (BiSKit) for large-area surface sampling. *Appl Environ Microbiol* **70**:7040-7045.
2. **Hodges, L. R., L. J. Rose, A. Peterson, J. Nobel-Wang, and M. J. Arduino.** 2006. Evaluation of a macrofoam swab protocol for the recovery of *Bacillus anthracis* spores from a steel surface. *Appl Environ Microbiol* **72**:4429 - 4430.
3. **Rose, L. J., L. Hodges, H. O. O'Connell, and J. Nobel-Wang.** 2011. National validation study of a cellulose sponge wipe-processing method for use after sampling *Bacillus anthracis* spores from surfaces. *Appl Environ Microbiol* **77**:8355-8359.

4. **Valentine, N. B., M. G. Butcher, Y. F. Su, K. H. Jarman, M. Matzke, B. J. Webb-Robertson, E. A. Panisko, B. A. B. Seiders, and K. L. Wahl.** 2008. Evaluation of sampling tools for environmental sampling of bacterial endospores from porous and nonporous surfaces. *J Appl Microbiol* **105**:1107-1113.
5. **Edmonds, J. M., P. J. Collett, E. R. Valdes, E. W. Skowronski, G. J. Pellar, and P. A. Emanuel.** 2009. Surface sampling of spores in dry-deposition aerosols. *Appl Environ Microbiol* **75**:39-44.
6. **Rose, L., B. Jensen, A. Peterson, S. N. Banerjee, and M. J. Srdino.** 2004. Swab materials and *Bacillus anthracis* spore recovery from nonporous surfaces. *Emerg Infect Dis* **10**:1023-9.
7. **Budowle, B., S. E. Schutzer, M. S. Ascher, R. M. Atlas, J. P. Burans, R. Chakraborty, J. J. Dunn, C. M. Fraser, D. R. Franz, T. J. Leighton, S. A. Morse, R. S. Murch, J. Ravel, D. L. Rock, T. R. Slezak, S. P. Velsko, A. C. Walsh, and R. A. Walters.** 2005. Toward a system of microbial forensics: from sample collection to interpretation of evidence. *Appl Environ Microbiol* **71**:2209-13.
8. **Budowle, B., S. E. Schutzer, J. P. Burans, D. J. Beecher, T. A. Cebula, R. Chakraborty, W. T. Cobb, J. Fletcher, M. L. Hale, R. B. Harris, M. A. Heitkamp, F. P. Keller, C. Kuske, J. E. Leclerc, B. L. Marrone, T. S. McKenna, S. A. Morse, L. L. Rodriguez, N. B. Valentine, and J. Yadev.** 2006. Quality sample collection, handling, and preservation for an effective microbial forensics program. *Appl Environ Microbiol* **72**:6431-8.
9. **Budowle, B., S. E. Schutzer, A. Einseln, L. C. Kelley, A. C. Walsh, J. A. Smith, B. L. Marrone, J. Robertson, and J. Campos.** 2003. Public health. Building microbial forensics as a response to bioterrorism. *Science* **301**:1852-3.

Alexeter Collection Swab™

Website: <http://www.alexeter.com/biow/index.asp>

Contact: Tom Fryzel (tfryzel@alexeter.com)

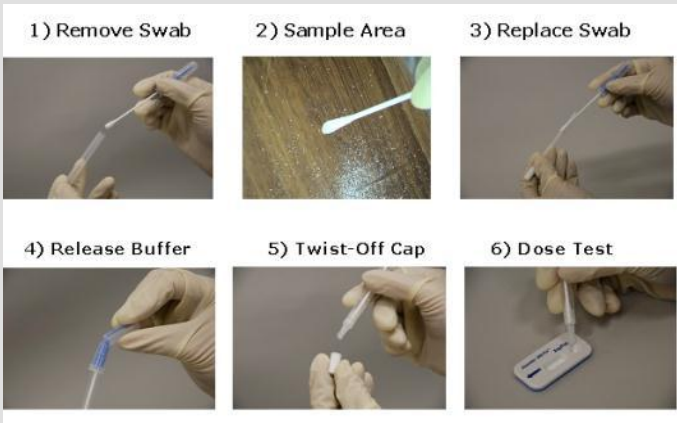
Phone: (877) 591-5571

Technology Summary

The Alexeter Collection Swab™ combines onsite sampling of biological agents and sample preparation for field-testing by immunoassay or PCR-based assay. The device includes a Dacron®-tipped swab, self-contained test buffer, pre-filter, and transfer sample dropper—all in one device. The all-in-one device minimizes sample transfer operations and is provided in a convenient single package.

Compatibility with Biodetection Assays

The Alexeter Collection Swab™ is designed to be used with Alexeter Bio-Detect immunoassay test strips. According to the company, the collection swab is compatible with PCR-based detection.



1) Remove Swab 2) Sample Area 3) Replace Swab

4) Release Buffer 5) Twist-Off Cap 6) Dose Test

Overview of the Alexeter swabbing protocol

Performance Summary

Immunoassay compatible? Yes
PCR compatible? Yes
Submersible decontamination? Yes
Cost: \$75/box of 25
Shelf-life: 2 years

References

No publicly available, peer-reviewed literature references were found for this product.

ASD Biosystems Sample Collection and Recovery Device

Website: <http://www.asdbiosystems.com/p1.htm>

Contact: ASD BioSystems, Inc. (info@asdbiosystems.com)

Phone: (804) 545-3102

Technology Summary

The ASD Biosystems Sample and Collection Recovery Device (SCRD) enables collection, transport, recovery, and retention of a specimen in a barcoded tube. The collection buffer is determined by the end-user. The collection device is a conical tube containing a buffer solution with a swab attached to the lid. The company offers four standard collection buffers (Tris with 1% pluronic acid, phosphate buffered saline (PBS), Water, or PBS with 0.1% Tween-20), all of which are compatible with immunoassay or PCR-based detection systems. After a sample is taken, the swab is rinsed in buffer, and the sample assayed using the detection system of choice.

Compatibility with Biodetection Assays

These collection devices can be used with immunoassay or PCR-based systems. Most of the available standard buffers offered by the company are compatible with both immunoassay and PCR-based detection.

References

No publicly available, peer-reviewed literature references were found for this product.



SCRD Kit

Performance Summary

Immunoassay compatible? Yes

PCR compatible? Yes

Submersible decontamination? Yes

Cost: \$12 per unit when purchasing 500 units

Shelf-life: 12 months

New Horizons Diagnostics SWIPE™ Kits

Website: http://www.nhdiag.com/profile_one.shtml

Contact: Larry Loomis (larryl@nhdiag.com)

Phone: (443) 543-5755

Technology Summary

New Horizons Diagnostics SWIPE™ kits are prepackaged, sterilized sampling kits for the HAZMAT community. SWIPE™ kits are available in five different formats, all of which contain a slide lock bag for dunkable decontamination, if necessary, to remove the collected sample from the hot zone. SWIPE™ I (\$23.10 each) is a large surface collection kit that comes with 25 mL of Aseptisol™ collection fluid and an adenosine triphosphate (ATP)–free sponge. SWIPE™ II (\$23.10 each) is a small-surface or powder collection kit, which includes a sterile spatula, collection tube, and two sterile swabs. SWIPE™ III (\$13.20 each) is a liquid collection kit with two sterile 10 mL syringes. SWIPE™ IV (\$18.15 each) is an aerosol on filter collection kit containing a sterile collection tube, 25 mL of Aseptisol™ collection fluid, and sterile forceps. SWIPE™ Sample Processing Kit (SPK) (\$17.33 each) is a kit designed to be used for sample dilution or pre-filtration of turbid samples outside the hot zone and includes syringes, collection tubes, 25 mL of Aseptisol™ fluid, and a biohazard bag for collection of waste.



SWIPE™ II Kit for powder collection on a small-surface

Performance Summary

Immunoassay compatible? Yes

PCR compatible? Yes

Submersible decontamination? Yes,
included slide lock bag in every kit

Cost: Varies, see text

Shelf-life: 18 months

Compatibility with Biodetection Assays

These collection devices can be used with immunoassay or PCR-based detection systems.

References

No publicly available, peer-reviewed literature references were found for this product.

TRIARII Scientific: LiNK2[®]

Website: <http://www.tri-sci.com/index.html>

Sales Contact: Jim Trecek (jtrecek@tri-sci.com)

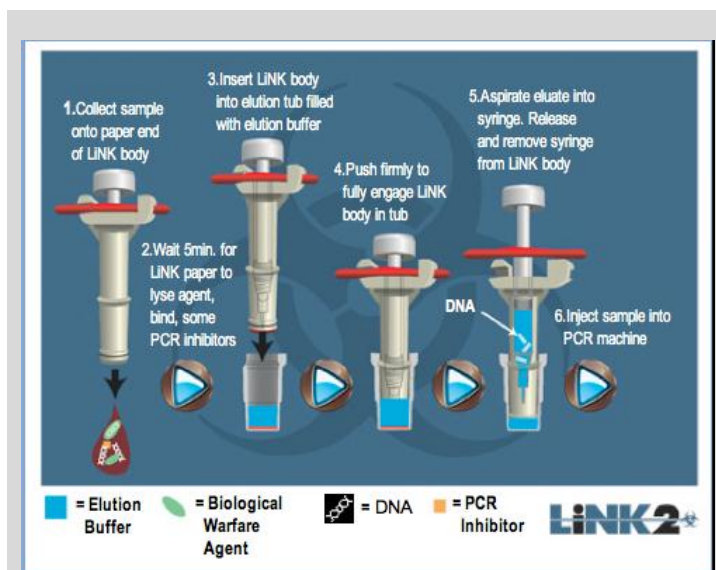
Technical Contact: Ryan Deady (ryan.deady@qckslvr.com)

Phone: (918) 760-5533

Technology Summary

The LiNK2[®] is a sample-processing system specifically designed to prepare samples (wet or dry) for PCR-based detection. The system is used to collect, lyse, and purify biological samples, removing potential PCR inhibitors in the process. Removing PCR inhibitors may be critical for some sample types to reduce false negative results due to inactivation or inhibition of the enzyme reagents in the PCR assay, which may cause a false negative result.

The Massachusetts Institute of Technology Lincoln Laboratory and the Naval Medical Research Center have evaluated this sample-processing system. The results of these evaluations are reported by the manufacturer along with the conclusion that PCR assays using the LiNK2[®] collection solution perform at levels comparable to standard laboratory sample cleanup processes. The PCR systems evaluated in combination with LiNK2[®] sample preparation included the RAZOR[®] EX (BioFire Diagnostics), LightCycler[®] (Roche), and 7900 Fast Real-Time PCR System (Applied Biosystems).



Overview of LiNK2[®] sampling method

Performance Summary

Immunoassay compatible? No

PCR compatible? Yes

Submersible decontamination? Can be placed back into re-sealable pouch for submersible decontamination

Cost: \$55/kit

Shelf-life: 2 years

Compatibility with Biodetection Assays

The LiNK2[®] system is designed to purify deoxyribonucleic acid (DNA) for PCR-based assays. This system is not recommended for immunoassays, because the sample processing may disrupt and/or remove the immunoassay targets.

References

No publicly available, peer-reviewed literature references were found for this product.

QuickSilver Analytics

Website: <http://chembiokits.com>

Contact: Ryan Deady (ryan.deady@qukslvr.com)

Phone: (410) 676-4300

Technology Summary

QuickSilver Analytics, Inc. is ISO 9001-2008 registered and a Center for Veterans Enterprise-verified Service Disabled Veteran-Owned Small Business. The company specializes in sampling kits for the HazMat community. Several biological sampling kits with slightly different utility are reviewed here. All kits use PBS buffer for biological sampling, which is compatible with immunoassays and PCR-based systems. In addition, sample containers can be dunked for decontamination after sample collection.

- **S3™ Bio Sampler** (\$22.56 each). The S3™ Bio Sampler is designed to collect biological samples from potentially contaminated surfaces onto a PBS buffer-moistened sterilized sponge for field or laboratory analysis.
- **Small Area Sampling (SAS™) Kit** (\$17.96 each). The SAS™ Kit is intended to sample small areas. The kit includes two swabs that can be moistened with the provided buffer (4 mL PBS) for sampling of powders or used directly for liquids. The swab can be broken off into the buffer bottle, placed into the 50 mL centrifuge tube, and shipped in the plastic bag provided for transport to a lab for analysis. A chain-of-custody form is also included. This kit has a 4-year shelf-life from the date of manufacture.
- **Biological Sampling Kit (BiSKit™) – Large Area Sampling Kit** (\$31.50). The BiSKit™ is designed to sample 1 m² of surface for bacteria, viruses, and toxins and to solubilize the sample in PBS buffer with sufficient volume (15 mL) for extensive testing and archiving. The kit has been designed to be easy to use in Level A protective gear and to enable transport of a sample without leaking. The lid of the unit has a foam material attached for collecting either a wet or a dry sample. After sampling, the lid is screwed back on and the unit can then be removed from the area for later testing or used for immediate analysis. This kit has a 2-



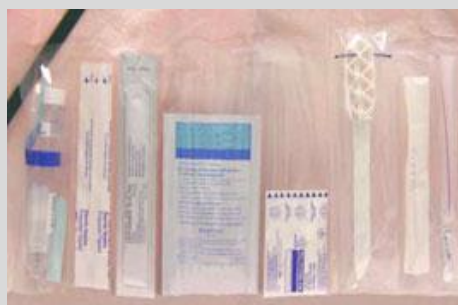
S3™ Bio Sampler



SAS™ Kit



BiSKit™



Mini Push Pack™ Sampling Kits

year shelf-life from date of manufacture.

- Mini Push Pack™ Sampling Kits:
 - Wipe Sampling Kit (\$51.30)
 - Biological Sampling Kit (\$36.70, pictured)
 - Liquid Sampling Kit (\$84.50)
 - Solid Sampling Kit (\$52.50)

These kits are highly compact and portable single-use units that contain the tools and supplies needed to take one sample per pack. All of the components are heat sealed into one package that is notched for easy opening. Each package is held together with a color-coded strap used to differentiate each type of sampling kit. Once sampling is complete, all of the waste can be placed on the absorbent liner, rolled up, and secured with the strap for disposal.

- QSA Model 102™ Full FAC (\$2961.80). This is a large sampling kit developed and used by the Edgewood Chemical and Biological Center (ECBC) Forensic Analytical Center (FAC) Mobile Labs Team. The kit (not shown) has materials for obtaining 27 total samples for chemical/biological/radiological/explosives testing.
- B2C™ Bulk Sample Collection Kit - SWB60013 (\$26.71). This kit meets Method A of the ASTM Standard E 2458-10 for bulk sample collection and packaging of visible powders suspected of being biological agents from nonporous surfaces.



Compatibility with Biodetection Assays

All kits use a PBS buffer for solubilizing collected samples; this buffer solution is compatible with both PCR-based detection systems and immunoassays.

References

Evaluation of the Biological Sampling Kit (BiSKit) for large-area surface sampling. Appl Environ Microbiol (2004) 70:7040-7045. This publication describes experiments performed to evaluate the BiSKit. As a result of the larger area that could be sampled with the BiSKit compared to swabs, 10 times more spores were recovered by the BiSKit® than swabs. The BiSKit® system had a sensitivity of detection of 42 ± 5.8 CFU/m² for wet sampling and 100.5 ± 10.2 CFU/m² for dry sampling and a recovery efficiency ranging from 11 to 18%.

3.0 Non-Specific Biological Tests

The goal of non-specific biological tests is to simply detect the presence of biological material in a sample. Typically these tests detect the presence of proteins, DNA, or ATP. Proteins and DNA are found in all cells, including spores and bacterial cells. Biological toxins, like ricin and botulinum toxin, are typically protein-based, but can be associated with DNA if the material is crudely prepared from cells. ATP is a metabolite found in living cells. Fourier Transform Infrared (FT-IR) spectroscopy is a common analytical tool that provides information about the sample properties by matching the spectral fingerprint of the sample to a library containing spectral fingerprints for thousands of compounds. Some FT-IR spectrometers include screening for biological samples, with a clear indication cautioning that the sample may be of biological origin.

While non-specific biological tests are relatively rapid and inexpensive, they should be used as a screening tool in conjunction with more specific tests. In general, these tests have low specificity and sensitivity, although the ATP test does have a reported limit of detection several orders of magnitude lower than immunoassays. These tests detect a broad range of biological materials, and therefore do not confirm the presence of a specific agent.

Protein Test

- Detects any type of protein (including milk proteins in coffee creamer and powdered infant formula)
- Protein tests may also include a pH test (biological material is typically neutral in pH)
- Easy to use (add sample, mix, and visually read)
- Operator manually reads color change for protein and pH
- Sample to answer in about 5 minutes
- Detection limit: 10 to 100 million *B. anthracis* spores (equivalent to about 1000 to 10,000 infectious doses, but still a barely visible amount of powder, <1 mg)
- Typical assay cost: ~\$40
- Typical shelf-life: 12 months
- Examples:
 - Biocheck® (20/20 BioResponse)
 - INDIPRO (Macherey-Nagel)

DNA Test

- Detects any type of DNA (e.g., human, plant, animal)
- Easy to use (add sample, mix, and read)
- Requires a fluorescence optical reader
- Sample to answer in about 5 minutes
- Detection limit: 1 to 10 billion *B. anthracis* spores (equivalent to about 1 to 10 million infectious doses and a readily visible amount of powder, approximately 1 to 10 mg)

- Instrument cost (fluorometer): \$12,000
- Typical assay cost: \$200
- Typical shelf-life: 12 months
- Example: Prime Alert[®] (GenPrime)

ATP Test

- Tests if any cell is present and alive
- Note, for spores an additional step (~15 minutes) must be performed prior to detection to stimulate the spore to convert to a vegetative (cellular) state to enable detection
- Requires a reader (luminometer)
- Sample to answer is about 20 minutes
- Detection limit: ~10,000 *B. anthracis* spores (approximately one infectious dose; amount not visible by eye)
- Luminometer reader cost: \$5000
- Typical assay cost: ~\$5
- Shelf-life: 12 months
- Example: Profile[®] 1 (New Horizons Diagnostics)

FT-IR Spectroscopy

- These systems were initially designed to rapidly identify chemicals. Proteins (contained in most biological materials) also give a defined FT-IR spectrum and *should* be detected in samples containing at least 10% protein.
- FT-IR systems have not been extensively tested as a screening tool for biological material in suspicious powders. In fact, Thermo Scientific (formerly Ahura) does not recommend the use of their system for this purpose.
- Chemicals are identified by comparing their spectrum to a library containing known chemical spectra. If a chemical's spectrum is not in the library, it will remain unidentified.
- Moderate ease-of-use; mixtures can be difficult to interpret
- Sample to answer in <5 minutes
- Detection limit: ~10% protein content in sample (No studies have been performed to determine the sensitivity in terms of number of spores).
- High instrument and upgrade costs (\$35 to 95K)
- No consumables
- Examples: HazMatID[™] Elite and HazMatID Ranger[™] (Smiths Detection); TruDefender[™] (Thermo Scientific).

HazTech Systems: KT7001 HAZCAT WMD Kit

Website: <http://www.hazcat.com>

Contact: Mark Parrish (mparrish@hazcat.com)

Phone: (800) 543-5487 (ext 207)

Technology Summary

The KT7001 HAZCAT WMD Kit consists of panels of chemical and biochemical assays to identify unknown substances. The kit is specifically for WMD detection and classification and consists of two Pelican™ cases containing evidence collection tools and materials, chemical testing reagents, and biological agent testing assays. The included chemical tests can detect explosives and chemical weapons. A radiological meter capable of detecting alpha, beta, and gamma radiation is also included. The biological agent tests include separate assays for detecting amino acids/proteins: Alexeter RAID™ 8 immunoassay tickets for detecting anthrax, plague, tularemia, brucellosis, orthopox, and botulinum, ricin, and SEB toxins; and other screens for non-biological materials and pesticides. The kit includes materials sufficient for responding to ten incidents.

Demonstrated Biodetection Assays

The kit detects a wide variety of potential hazardous substances, including radiation, chemicals (e.g., nerve agents and pesticides), and pathogens (e.g., anthrax). The kit includes general protein and pH assays as well as a standalone multiplexed immunoassay for anthrax, botulinum, SEB, and ricin toxins; plague; tularemia; brucellosis; and orthopox (Alexeter RAID™ 8).

References

No publicly available, peer-reviewed literature references were found for this product.



The KT7001 HAZCAT WMD Kit

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes

Assay time: ~10 minutes

Detection limit (reported by manufacturer):

10,000 anthrax spores/mL; 6 ng/mL ricin

Required sample preparation? Moderate—many single tests are required

Automatic results display? Operator reads/interprets results

Unit weight: ~28 lb

Power: Standard batteries

System cost: \$4910.17

Additional costs: Assays can be restocked individually; prices vary

Shelf-life: Kit components vary; 1 to 5 years

GenPrime: Prime Alert®

Website: <http://www.genprime.com/>

Sales Contact: Ken Klein (ken.klein@smithsdetection.com); (302) 353-0909

Technical Contact: Darby McLean (dmclean@genprime.com); (866) 624-9855

Technology Summary

The Prime Alert® System is designed to test for biological material in a white powder sample based on the presence of large amounts of DNA/ribonucleic acid (RNA) or proteinacious biothreat toxins. This system is distributed through Smiths Detection.

The Prime Alert® System contains two components: a microbe screen (an assay that detects DNA) and a toxin screen (lateral flow immunoassays for specific toxin detection). The advantage of the microbe screen is that it is a broad screen for any type of microbe sample, not just anthrax. However, the test is not specific and only indicates if the sample contains a large quantity of DNA. The DNA assay consists of a DNA binding dye that fluoresces only when bound to DNA. Fluorescence emission is read by a handheld fluorometer included with the system. A calibration standard is included with the kit and used to calibrate the fluorescent reader before each use. The assay takes approximately 5 minutes to run. The immunoassay toxin test is a standard lateral flow immunoassay that contains assays to detect ricin, botulinum neurotoxin serotype A, and SEB in a single cassette. The immunoassay is optimized to work with the microbe screen sample buffers, so both tests can be performed from a single solubilized sample.

Demonstrated Biodefense Assays

The Prime Alert® System microbe screen is intended to be used as a broad screen for biological material in visible powder samples.

The Prime Alert® System toxin screen is an immunoassay that detects ricin, SEB, and botulinum toxin. Testing by ECBC demonstrated that the Prime Alert® System was sensitive to 1 to 20 trillion *Bacillus anthracis* Sterne spores, which is significantly higher than the limit of detection reported by the manufacturer. The sensitivity was dependent on the number of times the spores were washed, as less



The Microbe and Toxin Screens included in the Prime Alert® System (note: the current toxin screen is a single three-agent cartridge).

Performance Summary

Anthrax detection? Yes, but not specifically, detection is based on high levels of generic DNA in sample

Ricin detection? Yes (separate immunoassay test)

Assay time: ~5 minutes for microbe screen; ~10 minutes for immunoassay toxin screen

Detection limit (based on published literature):
microbe screen: 100 million to 10 trillion spores/mL;
immunoassay: 400 ng/mL (50 ng/test) ricin and botulinum neurotoxin

Required sample preparation? Minimal

Automatic results display? Yes, microbe test; user interprets presence/absence of a line, toxin immunoassay

Unit weight: 1 lb (reader); 12 lb (complete kit)

Power: 4 AAA batteries (2000+ readings)

System cost: Starter Kit: \$12,000; includes fluorometer and supplies for five complete screens and two training kits; Test Kits: \$200 (microbial and toxin screens); Microbial Screen: \$90

Additional costs: None

Shelf-life: 12 months

washing allowed for better detection of extraneous DNA present on the surface of spores (see reference below). In contrast, an evaluation performed by Battelle in 2002 (report available upon request from GenPrime, see References) found the assay sensitive down to 100 million spores (determined using *B. subtilis* spores). This study also found that samples containing unbleached flour will give a false-positive result.

References

An evaluation of suspicious powder screening tools for first responders. J Hazard Mater. (2009) 172 (2-3), 559-65. This study reports the detection limit for anthrax spores to be between 1 to 10 trillion colony forming units (CFU) depending on how stringently the spores were washed (the more washes, the more difficult the spores were to detect). Note: the limit of detection reported by the manufacturer is between 100,000 and 1 million CFU/mg using lyophilized spores.

Final Report for Evaluation of the GenPrime Prime Alert™ Powder Assessment Kit (Reference No. CPO50297; not peer reviewed). Prepared by Battelle Memorial Institute. (April 2002). This evaluation of the Prime Alert™ kit was sponsored by GenPrime. Document available upon request from GenPrime.

20/20: BioCheck® Powder Screening Kit

Website: <http://www.biocheckinfo.com>

Contact: Barry Cohen (sales@2020gene.com)

Phone: (240) 453-6339 (ext 103)

Technology Summary

The BioCheck® Powder Screening Kit is a tool to screen for biological material in visible powder samples. Note: the test is not designed for environmental sampling (e.g., soil). A sample is collected with the provided swab for protein analysis, added to a liquid-containing tube, and allowed to incubate for 5 minutes. If the sample is positive for protein, the solution in the tube turns blue. If the protein assay is negative, the results are validated by inserting the provided control swab into the protein test tube—if the assay is working correctly, this swab should turn the solution blue. A positive protein result is a tentative indication of the presence of a biological agent or toxin. The pH tube determines if the sample is acidic, basic, or neutral: it will turn pink if the sample is acidic, purple if the sample is basic, and have no color change or turn slightly yellow if the sample is neutral. The pH of biological samples is typically neutral. Samples with an acidic pH may cause the protein test to fail. If a BioCheck® test is positive, additional tests should be performed to determine the presence of a biological agent of concern or if the sample merely contains a harmless protein-containing substance (e.g., baby formula, powdered milk).

Demonstrated Biodefense Assays

The BioCheck® Powder Screening Kit has been independently evaluated using a variety of *Bacillus* species spore preparations (e.g., washed spores, unwashed spores, spores with silica). Consistent detection of protein in all samples containing ≥ 1 million spores was reported (evaluation by Battelle, weblink to report provided in References). The BioCheck® Powder Screening Kit was also evaluated at ECBC (see reference listed below), where positive protein detection was found to depend on whether *B. anthracis* Sterne spores were washed or not (i.e., the method of spore preparation). In the ECBC evaluation, the average detection levels were 10 million *B. anthracis* Sterne spores and 100 μg of ricin. Positive protein detection of a *Yersinia pestis* preparation was reported at 10 million cells.



The BioCheck® Powder Screening Kit

Performance Summary

Anthrax detection? Yes, but not specifically; detection is based on high levels of generic protein in sample

Assay time: ~5 minutes

Ricin detection? Not specific, based on high levels of generic protein in sample

Detection limit (reported by manufacturer):

80 million CFU/mL anthrax spores; 20 μg ricin

Required sample preparation? No

Automatic results display? User interprets color change

Unit weight: Negligible

Power: None

System cost: \$687.50 for 25 test kits

Additional costs: Training Package (optional)—includes training presentation on CD, detailed information on testing, and training powders

Shelf-life: 14 months

References

An evaluation of suspicious powder screening tools for first responders. J Hazard Mater. (2009) 172 (2-3), 559-65. This study reports the detection limit for anthrax spores to be between 10 to 100 million CFU depending on how stringently the spores were washed (the more washes, the harder the spores were to detect). Note: these studies were performed with spore samples added directly to the kit solutions, thus detection limits are not reported per milliliter. However, the reported results are within range of the detection limit reported by the manufacturer.

20/20 GeneSystems Bio-Identification Kit Testing Summary Report: Tier I and II. Prepared by Battelle. (September 2002; not peer reviewed). This evaluation of the Prime Alert™ kit was sponsored by 20/20. http://www.biocheckinfo.com/Battelle_Testing.pdf

Macherey-Nagel Indipro: Protein Test Strips

Website: <http://www.mn-net.com/tabid/10441/default.aspx>

Contact: Henry Medollo (U.S. supplier at CTL Scientific Supply Group); (hmedollo@ctlscientific.com)

Phone: (631) 242-4249

Technology Summary

Like the BioCheck[®] Kit, these strips detect the presence of protein. The standard protocol for use is to first moisten the test strip with one drop of solution “indipro-1” and then wipe the test strip over the test surface. Next, the strip is developed by applying a drop of “indipro-2” solution directly to the strip. A color change (yellow to green) indicates the presence of protein.

Demonstrated Biodeflection Assays

The indipro test is primarily marketed to the food and restaurant industries for detecting protein contamination on work surfaces and utensils. However, being an assay for generic proteins, this test can also be used in a manner similar to the 20/20 BioCheck[®] assay to determine if a suspicious powder contains protein. The manufacturer claims a limit of detection of 50 µg protein. This detection limit was determined using a bovine serum albumin protein standard.

References

No publicly available, peer-reviewed references were found for this product.



The Indipro Test Kit

Performance Summary

Anthrax detection? Yes, but not specifically, detection is based on high levels of generic protein in sample

Assay time: ~5 minutes

Ricin detection? Not specific, based on high levels of generic protein in sample

Detection limit (reported by manufacturer): 50 µg protein

Required sample preparation? Minimal

Automatic results display? User interprets color change

Unit weight: Negligible

Power: None

System cost: \$103/box of 60 strips

Additional costs: None

Shelf-life: 12 to 24 months (depending on date of manufacture)

New Horizons Diagnostics PROFILE[®] 1: ATP Test

Website: http://www.nhdiag.com/profile_one.shtml

Contact: Larry Loomis (larryl@nhdiag.com)

Phone: (443) 543-5755

Technology Summary

The PROFILE[®] 1 kit detects the presence of living bacterial cells by measuring the amount of ATP in a sample. ATP is a cellular metabolite present in all living cells. The amount of ATP in a sample is typically proportional to the number of living cells. The test can distinguish bacteria from human cells through the use of sample preparation methods that release ATP selectively from microbial cells. The system has two main components: a microluminometer to read ATP-induced luminescence and a “filtravette” (a filter device and cuvette combined) to concentrate cells from a sample solution and remove chemical contaminants that may interfere with the test. Samples and solutions are processed through the filtravette using an empty syringe. After the sample is processed, a solution is applied to remove non-microbial sources of ATP. Then, a microbial lysis solution is added to the filtravette, followed by a luciferase solution that produces light in the presence of ATP. After pipette mixing, the filtravette is placed in the reader and the intensity of emitted light (luminescence) is recorded. Ricin and botulinum toxin are not detected by this method.

Spores do not contain high levels of ATP and are essentially undetectable using the PROFILE[®] 1 kit unless an incubation step is performed to initiate cell growth. When analyzing a suspected anthrax spore powder, the sample must be incubated in growth media for 15 minutes and two ATP measurements taken: one prior to incubation, and one after incubation. Note: using this method, powders containing yeast cells or Dipel dust (i.e., biological insecticide) may also give a positive result.

Demonstrated Biodefense Assays

The PROFILE[®] 1 is primarily marketed to the food safety industry as an alternative to culture methods to rapidly determine if a sample contains viable organisms. The system has been evaluated specifically for anthrax detection at ECBC (see reference below). This evaluation reported a limit of detection of 10,000 anthrax spores and notes that flour and talcum powder produce false-positive results. The manufacturer reports these false-positive reactions are eliminated by using the somatic cell releasing reagent before testing final samples in the filtravette. Since the ECBC evaluation, the manufacturer indicates that it has made improvements to the microluminometer, decreasing the detection limit 10-fold.



PROFILE[®] 1 Kit for detection of live cells

Performance Summary

Anthrax detection? Yes, but not specifically; detection is based on presence of ATP in sample

Ricin detection? No

Assay time: ~15 minutes (longer growth times can be used to improve sensitivity)

Detection limit (reported by manufacturer):
10,000 CFU/mL

Required sample preparation? Yes

Automatic results display? User interprets numerical readout

Unit Weight: <1 lb

Power: 9V battery (1000+ reads) or AC

System cost: Profile[®] 1 system: \$5000 (includes carrying kit, microluminometer, and pipettor),
Reagent kit: \$450 (reagents and materials for 100 tests)

Additional costs: None

Shelf-life: 12 months (some reagents require refrigeration)

References

An evaluation of suspicious powder screening tools for first responders. J Hazard Mater. (2009) 172 (2-3), 559-65. The limit of detection (LOD) reported by this study was 10,000 CFU/mL, independent of the stringency of spore washing. Flour and talcum powder produced false-positive results, but may have resulted from bacterial contamination of those powders. A procedure modification made by the manufacturer may have addressed these issues.

Smiths Detection: HazMatID™ 360

Website: <http://www.smithsdetection.com/hazmatid360.php>

Contact: Chris Lins (chris.lins@smithsdetection.com)

Phone: 770-664-8723

Technology Summary

The HazMatID™ 360 is a hand portable FT-IR spectrometer for solid and liquid samples. The device includes an integrated press to ensure high-quality data collection of solid samples and an integrated well for liquid samples. A finger or stylus can be used to control the system via a touchscreen. The software provides enhanced mixture analysis and classification tools and PEAC® decision-support software provides detailed information regarding the management of hazardous chemicals. The system can identify over 32,000 substances, including many white powders. The system can operate in temperatures ranging from 19 to 122°F and in any level of humidity. A removable, rechargeable battery provides 2 hours of operation and recharges in 3 hours. The device can also use AC or automobile power. Data can be stored on removable USB, flash, floppy, or CD drives. The system is mouse and keyboard compatible and runs a Windows®-based operating system. Available options include: ExtractIR (a system for removing chemicals from aqueous solutions for improved identification), wireless Bluetooth capability, additional spectral libraries, and a software upgrade.

Demonstrated Biodetection Assays

FT-IR is a well-established technology widely used by hazardous materials teams across the nation to identify chemical solids, liquids, and powders. Biological materials are generically identified primarily based on protein content. Though the analysis of mixtures presents a challenge, the HazMatID™ 360 alerts the user when a sample has a protein content of approximately 10% or more, indicating a sample of potential biological origin.

References

No publicly available, peer-reviewed references were found for this product. However, the manufacturer has published the following article in the Proceedings of the SPIE (not peer reviewed): Arno, J., et al. "Advances in Handheld FT-IR Instrumentation" Proc. SPIE 8374. <http://proceedings.spiedigitallibrary.org/proceeding.aspx?articleid=1354102>



The HazMatID™ 360 is a hand portable FT-IR with integral sample press

Performance Summary

Anthrax detection? Yes, but not specifically; detection is based on high levels of generic protein in sample

Ricin detection? Yes, but not specifically; detection is based on high levels of protein in sample

Assay time: ~5 minutes

Detection limit (reported by manufacturer): Sample must contain approximately 10% protein

Required sample preparation? No, use integrated solid sample press or liquid well.

Automatic results display? Yes

Unit weight: 23 lb

Power: Internal rechargeable battery, AC, or automobile cigarette lighter

System cost: \$53,000

Additional costs: ExtractIR, Bluetooth, additional spectral libraries, software upgrade; Optional wireless capability to interface with Responder RCI™ Raman spectrometer

Shelf-life: N/A

Smiths Detection: HazMatID™ Ranger

Website: http://www.smithsdetection.com/hazmatid_ranger.php

Contact: Chris Lins (chris.lins@smithsdetection.com)

Phone: 770-664-8723

Technology Summary

The HazMatID™ Ranger is a FT-IR designed for handheld, backpack, or robot portability and ease of use. Samples are measured by firmly pressing the diamond attenuated total reflection (ATR) sensor head directly onto a liquid or solid sample. Spectral results and a list of probable substances are displayed on the attached touchscreen personal digital assistant (PDA). The ruggedized device, but not the PDA, can be decontaminated via dunking and can withstand 40G of shock. The instrument can operate in any level of humidity and at temperatures from 19 to 122°F. The system battery runs for >3 hours and the PDA battery runs for >8 hours. The standard library contains spectra of >4400 materials, and the optional library contains spectra of >32,000 materials. Another additional option is wireless capability that allows communication with a laptop computer for advanced combined analysis with the Responder RCI™ portable Raman spectrometer. In addition, for difficult samples (i.e., small sample amounts or samples located on irregular surfaces), the PDA can be removed and the sample placed on an optional solids press accessory for measurement.

Demonstrated Biodetection Assays

While FT-IR is a well-established technology, use of handheld FT-IR is relatively new. Both the handheld Ranger and the hand portable HazMatID™ 360 have extensive libraries used to identify materials, including most known white powders. Biological materials are identified generically, primarily based on their protein content. Though the analysis of mixtures presents a challenge, the HazMatID™ Ranger alerts the user when a sample has a protein content of approximately 10% or more, indicating a sample of potential biological origin.

References

No publicly available, peer-reviewed references were found for this product. However, Smiths Detection offers an application brief that discusses how the HazMatID™ Ranger compares to its hand



The HazMatID™ Ranger is a handheld FT-IR

Performance Summary

Anthrax detection? Yes, but not specifically;
detection is based on high levels of generic protein in sample

Ricin detection? Yes, but not specifically;
detection is based on high levels of protein in sample

Assay time: ~5 minutes

Detection limit (reported by manufacturer):
Sample must contain approximately 10% protein

Required sample preparation? No. Sensor tip is touched directly to sample

Automatic results display? Yes

Unit weight: 6.5 lb

Power: Internal battery (>3 hours of operation)

System cost: \$35,000

Additional costs: Optional wireless capability to interface with Responder RCI™ Raman spectrometer; optional >32,000 material library (vs. standard >4400)

Shelf-life: N/A

portable predecessor, the HazMatID™360.

http://www.smithsdetection.com/media/Application_Brief_86_-_Handheld_FT-IR.pdf

In addition, the manufacturer has published an article in the Proceedings of the SPIE (not peer reviewed): Levy, D. “Advances in Portable FTIR Spectrometers for the field: the HazMatID Ranger” Proc. SPIE 7319. <http://proceedings.spiedigitallibrary.org/proceeding.aspx?articleid=777822>

Smiths Detection: HazMatID™ Elite

Website: <http://www.smithsdetection.com/HazMatIDElite.php>

Contact: Chris Lins (chris.lins@smithsdetection.com)

Phone: 770-664-8723

Technology Summary

The HazMatID™ Elite is the next generation FT-IR specifically designed for ease-of-use in the hot zone and extreme environments. The device includes an integrated press to ensure high-quality data collection on solid samples and an integrated well for liquid samples. A second touch-to-sample diamond ATR sensor is available for analysis of pooled liquid and surface films and to enable robotics applications. The 4.3-inch color display has high visibility in direct sunlight. Easy-to-use software and on-screen instructional graphics guide the user through essential operations. Automated mixture analysis software is claimed to simplify complex sample analysis. The device is designed to military standards (MIL-STD-810G) and can operate in temperatures ranging from -4 to 122°F and any level of humidity. A rechargeable lithium-ion battery provides 4 hours of operation. The device can also use disposable 123A batteries, as well as AC or automobile power. An embedded radio frequency (RF) modem enables line-of-sight communication of up to 1 km for data transfer and remote operation. A global positioning system (GPS) is also included. The device library includes 10,000 known spectra and user-defined libraries can be transferred from the HazMatID™ 360 hand portable unit. Using a command PC, an optional software package allows data management and spectral reprocessing against an upgraded library of 35,000 spectra. In addition, a RF repeater is available for extending the wireless range.

Demonstrated Biodefense Assays

While FT-IR is a well-established technology, use of handheld FT-IR is relatively new. The HazMatID™ Elite, like the hand portable HazMatID™ 360, has an extensive library used to identify materials including most known white powders. Biological materials are generically identified primarily based on their protein content. Though the analysis of mixtures presents a challenge, the



The HazMatID™ Elite is a handheld FT-IR with integral sample press

Performance Summary

Anthrax detection? Yes, but not specifically; detection is based on high levels of generic protein in sample

Ricin detection? Yes, but not specifically; detection is based on high levels of protein in sample

Assay time: ~5 minutes

Detection limit (reported by manufacturer): Sample must contain approximately 10% protein

Required sample preparation? No, touch sensor tip directly to sample or use integrated solid sample press or liquid well.

Automatic results display? Yes

Unit weight: 5 lb

Power: Internal rechargeable battery or disposable 123A batteries (4 hours of operation)

System cost: \$50,000

Additional costs: Optional software package for data reprocessing on a remote laptop PC using 35,000 spectra library; Optional RF repeater for increased wireless range

Shelf-life: N/A

HazMatID™ Elite alerts the user when a sample has a protein content of approximately 10% or more, indicating a sample of potential biological origin.

References

No publicly available, peer-reviewed references were found for this product. However, the manufacturer has published the following article in the Proceedings of the SPIE (not peer reviewed):
Arno, J., et al. “Advances in Handheld FT-IR Instrumentation” Proc. SPIE 8374.
<http://proceedings.spiedigitallibrary.org/proceeding.aspx?articleid=1354102>

Thermo Scientific (Formerly Ahura): TruDefender® FT and FTi

Website: <http://www.ahurascientific.com/chemical-explosives-id/products/trudefenderft/index.php>

Contact: Tom Keller (tom.keller@thermoscientific.com)

Phone: (480) 532-6171

Technology Summary

The TruDefender® FT and FTi are handheld FT-IR instruments for analyzing and identifying chemical substances, including white powders, explosives, narcotics, and chemical weapons. The FTi version adds wireless communication, allowing results to be transmitted via email or text message. *Thermo Scientific does not recommend the system for identification or detection of biological material due to the low sensitivity of FT-IR for accurately detecting biological materials in suspicious powders.*

A sample is analyzed by contacting the diamond ATR sensor head directly to a liquid or solid sample. A “sample crusher,” or sampling platform, is included for analyzing solid samples and volatile liquids. Once a sample is scanned, spectral results are compared to a library of known spectra. The current spectral library (version 1.5) contains over 10,000 spectra; users can also add custom spectra to the library. In addition, TruDefender® software uses an automated proprietary algorithm for analyzing mixtures of chemicals without manual spectral subtraction and subjective evaluation. The TruDefender® series is designed to be used in a hot zone wearing Level A gear. For decontamination, the unit can be immersed in water and household bleach (5% sodium hypochlorite).

Demonstrated Biodefense Assays

Thermo Scientific does not endorse these systems for detecting the presence of biological material in suspicious powders.

References

While several peer-reviewed publications discuss the TruDefender® system for chemical identification, no peer-reviewed publications were found for its use in detection and identification of biological materials.



The TruDefender® is a handheld FT-IR

Performance Summary

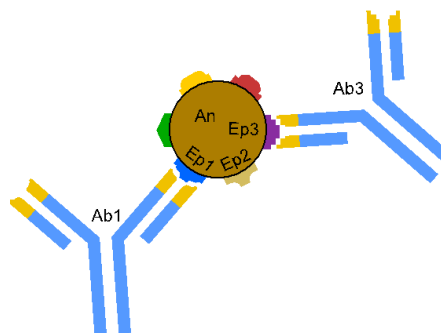
Anthrax detection? Not recommended
Ricin detection? Not recommended
Assay time: ~5 minutes
Detection limit (reported by manufacturer):
 Not determined
Required sample preparation? No, touch sensor tip directly to sample
Automatic results display? Yes
Unit weight: 2.9 lb
Power: Removable, rechargeable battery or 123A SureFire™ batteries (>4 hours operation)
System cost: \$45,000 (FT); \$46,500 (FTi)
Additional costs: Software/library updates are included with active support contracts
Shelf-life: N/A

4.0 Immunoassays

The goal of biodetection immunoassays is to differentiate and detect the presence of specific threats (pathogen and toxin) in a sample. These assays use antibodies, which are proteins designed (by nature or in the laboratory) to bind to a specific threat agent.

Most field-based immunoassays use a lateral flow assay (LFA) format similar to a home pregnancy test. An LFA includes an assay strip containing all the assay components encased in a plastic cartridge. The cartridge has a sample window where the sample is applied to the assay strip and a results window where the results are read. LFAs require samples to be in a liquid form, which typically necessitates a swab collection kit that solubilizes material from the swab into a buffer. After a sample is collected, approximately 5 or 6 drops (<0.5 mL) of sample solution are added to the sample inlet window. The sample solution is wicked across the strip and through a reagent zone that contains dye-labeled antibodies specific to the threat agent. Threat agent molecules in the sample bind to the colored antibody-dye as the sample passes through this zone. The sample continues to be wicked into a capture zone containing a second set of antibodies that bind the threat agent/antibody-dye complex. The secondary antibodies in the capture zone are immobilized in a line across the strip so that, as capture proceeds, a visible line (the test line) develops in the test window. Adjacent to the capture zone is a control zone that contains immobilized antibodies (the control line) that bind to the antibody-dye directly. For an assay to be considered positive, both the test and control lines must be visible. For an assay to be considered negative, only the control line should be visible.

Immunoassays are advantageous because they are relatively inexpensive, require little skill to use, and results can be obtained in only 5 to 15 minutes. However, the specificity and sensitivity of immunoassays vary depending primarily on the quality of the antibodies used in the assay. For example, some antibodies can bind very low concentrations of target threat agent resulting in a very sensitive assay. Other antibodies may bind the target molecule but also have some affinity for closely related molecules (like non-pathogenic relatives of *B. anthracis* that are found naturally in the environment). Furthermore, immunoassays are subject to false negatives resulting from interferents and highly concentrated samples. For example, if there are more target molecules than dye-labeled antibody molecules, unlabeled target molecules may bind to the test line and effectively block the labeled target molecules from binding. In this situation, the target line would be negative and the control line may or may not be positive (depending on the assay design). Typical detection limits for immunoassays range from 100,000 to 10 million spores or microbes.



Schematic drawing of two different antibodies (Ab1 and Ab3) binding to two different regions (Ep1 and Ep3) on a target molecule. (By Antibody.svg: Fvasconcellos Epitope.png: Schorschski derivative work: Marek M (Antibody.svg Epitope.png) [CC-BY-SA-3.0], via Wikimedia Commons).

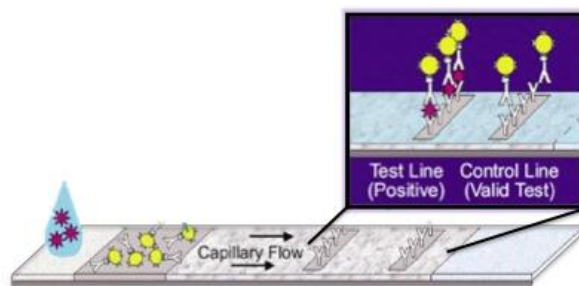


Illustration of a Typical Immunoassay. (By U.S. National Aeronautics and Space Administration (Lateral_Flow_Assay.jpg) [Public domain], via Wikimedia Commons)

Single Target Tests

- Assay cost: ~\$25 to 30
- Reader cost: \$5 to 10K (optional)
- Note: the reader improves sensitivity and accuracy and may help to identify a hook effect (excess target threat agent) condition
- Examples: BADD™ (Advnt), BioDetect™ (Alexeter), and many others

Multiple Target Tests

- 3- to 8-plex ticket
- Assay cost: \$70 to 100
- Optional reader for NIDS™: \$7.5K
- Examples: Pro Strips™ (Advnt), RAID™ (Alexeter), NIDS™ (Smiths Detection) and others

Automated Systems

- BIOSENSOR™ 2200R (MSA/QTL)
 - 2-plex (*Bacillus* spores, ricin toxin)
 - Detection limit ~10,000 *Bacillus* spores
 - Assay cost: \$50
 - Instrument cost: \$16K
- RAPTOR™ (Research International)
 - 4-plex
 - Assay cost: \$200
 - Instrument cost: \$49.5K

Advnt: BADD™ Biowarfare Agent Detection Devices

Website: <http://www.advnt.org/>

Contact: Kevin Borah (kevinb@advnt.org)

Phone: (888) 223-3269

Technology Summary

The Advnt Biowarfare Agent Detection Devices (BADD™) are LFAs for the detection of specific biothreats. These devices test for one agent/target at a time. Each test comes with an all-in-one swab kit that contains a swab, sample buffer, and pipette/dropper tube. Following sampling, the test is initiated by adding five or six drops (~0.2 mL) of sample to the sample inlet window. Results (presence/absence of lines in the test window) can be seen in 15 minutes; however, with concentrated samples a positive result may be obvious after only three minutes. The test produces one of three results: positive—target detected (two lines in the test window, one for the target and one for the internal positive control), negative—target not detected (single line at the positive control zone), and inconclusive—assay error (no lines, or a single line for the target but no control line).

Demonstrated Biodetection Assays

Individual tests are available for anthrax, plague, ricin, botulinum, and SEB. Advnt reports that the LOD of the anthrax assay ranges from 15,000 to 83,000 spores/mL depending on the strain tested. This equates to approximately 3000 to 16,600 spores if a five to six drop (~0.2 mL, according to the manufacturer) sample is applied. The LODs reported by the manufacturer for other biothreat targets are: 10 ng/mL for ricin, 33 ng/mL for botulinum A, 500 ng/mL for botulinum B, 100,000 organisms/mL for *Y. pestis*, 10 ng/mL for SEB, and 1.4 million CFU/mL for *F. tularensis*. Peer-reviewed studies (listed in the references) in 2003 and 2007 report higher detection limits; however, the manufacturer indicates these studies were performed with the first generation of the assay, which has been subsequently updated several times (2006, 2010, 2012/2013). A weblink to Advnt-sponsored performance specifications is provided in the References.

References

Performance Assessment of Three Commercial Assays for Direct Detection of *B. anthracis* Spores. Journal of Clinical Microbiology (2003) 3454–3455. This brief study evaluated the 1st generation



The Advnt BADD™ lateral flow immunoassay cartridges

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes

Assay time: ~15 minutes

Detection limit (reported by manufacturer):

15,000 to 83,000 anthrax spores/mL; 5 ng/mL ricin

Required sample preparation? Minimal

Automatic results display? User interprets

presence/absence of a line

Unit weight: Negligible

Power: Not required

System cost: \$245 (box of 10) or \$24.50 per test; hard case kit for \$838.50 contains a total of 30 assays and can be configured as ten tests for each of three different targets (i.e., anthrax, ricin, botulinum), six tests for each of five different targets (i.e., anthrax, ricin, botulinum, *Y. pestis*, SEB), or five tests for each of six different targets (i.e., anthrax, ricin, botulinum, *Y. pestis*, SEB, *F. tularensis*)

Additional costs: none

Shelf-life: 24 months (room temperature)

BADD™ anthrax assay (4th generation assay is expected to be released in 2013) and reported detection of 100,000 spores in three out of three tests; the assay could not detect 10,000 or less spores. The volume of the sample applied was not reported.

Evaluation of lateral flow assays for the detection of botulinum neurotoxin type A and their application in laboratory diagnosis of botulism. Diagnostic Microbiology and Infectious Disease (2007) 57 243–249. This study reports the detection limit of the 1st generation assay for botulinum neurotoxin type A to be 100 ng/mL using a 125 µL sample (currently Advnt recommends using a 0.2 mL sample (5 to 6 drops)). This study also found the assay detects both purified toxin and toxin in complex with its natural, non-toxic carrier proteins.

Performance Spec Sheets for Prostrips and BADD. Results reported by Advnt. (2012)
http://www.advnt.org/products/biowarfare/techlibrary/SpecSheet_PS_and_BADD.pdf

Advnt: Pro Strips™ Multi-Agent Detection Tests

Website: <http://www.advnt.org/>

Contact: Kevin Borah (kevinb@advnt.org)

Phone: (888) 223-3269

Technology Summary

The Advnt Pro Strips™ are basically the BADD™ assays bundled into a single cartridge. In the Pro Strips™ assay, one sample is automatically divided among five different tests (i.e., anthrax, ricin, botulinum, *Y. pestis*, and SEB). Following sample collection with the included all-in-one swab kit, approximately 10 to 11 drops (~0.4 mL) of the solution is added to the sample inlet window. Results are read in a separate window for each target tested. For a valid test, a control line must also be observed in each window. The recommended incubation time is 15 minutes, but results may be generated in as little as 3 minutes for concentrated samples.

Demonstrated Biodefense Assays

The Pro Strips™ cartridge contains assays for 5 targets: *B. anthracis*, ricin, SEB, botulinum toxin, and *Y. pestis*. The manufacturer asserts that because the assays are essentially the same as the BADD™ individual assays, they will have the same LODs and are reported as: 15,000 to 83,000 spores/mL (assuming a 0.4 mL sample is applied to cartridge this equates to 6000 to 33,200 spores), 10 ng/mL for ricin, 33 ng/mL for botulinum neurotoxin serotype A, 100,000 CFU/mL for *Y. pestis*, and 10 ng/mL for SEB. A weblink to Advnt-sponsored performance specification studies is provided in References.



The Advnt Pro Strips™ cartridge for anthrax, ricin, botulinum, plague, and SEB

Performance Summary

Anthrax detection? Yes
Ricin detection? Yes
Assay time: ~15 minutes
Detection limit (reported by manufacturer):
 15,000 to 83,000 anthrax spores/mL; 5 ng/mL ricin
Required sample preparation? Minimal
Automatic results display? User interprets presence/absence of a line
Unit weight: Negligible
Power: Not required
System cost: \$699.50 (box of 10)
Additional costs: None
Shelf-life: 24 months (room temperature)

References

No publicly available, peer-reviewed literature references were found for this product.

Performance Spec Sheets for Prostrips and BADD. Results reported by Advnt. (2012)
http://www.advnt.org/products/biowarfare/techlibrary/SpecSheet_PS_and_BADD.pdf

Alexeter: BioDetect™ Test Strips

Website: <http://www.alexeter.com/biow/index.asp>

Contact: Tom Fryzel (tfryzel@alexeter.com)

Phone: (877) 591-5571

Technology Summary

The Alexeter BioDetect™ Test Strips are standard single target/agent LFAs. Prior to 2005, Alexeter BioDetect™ strips were manufactured by Tetracore and identical to the BioThreat Alert® Test Strips. However, the current product is independent of Tetracore and includes different reagents. Each cartridge comes with a sampling kit that includes an integrated swab, buffer, and dispenser. To initiate the assay, a 3 to 5 drop sample (~150 to 250 µL) is dispensed into the sample window. After a 15-minute incubation, the test can be read either by eye or with the Alexeter Guardian™ Reader or Defender™ TSR (Test Strip Reader). There are two test lines in the test window: a control line in which a red line should always develop for a valid test and a sample line in which a red line appears if the target is detected. For a valid result, positive or negative, the control line must always be visible. For the toxin assays, the manufacturer recommends running an additional test with a 1:40 dilution of the sample to minimize the possibility of over-sampling (hook effect).

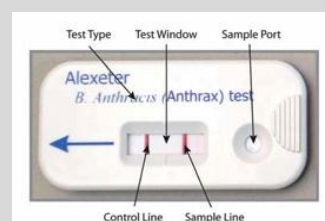
For increased accuracy and sensitivity, these test strips can be read with the Alexeter Guardian™ Reader or Defender™ TSR systems (available separately). The primary advantages of using either reader are an objective interpretation of the test result and enhanced detection sensitivity (reported by the vendor as approximately 10-fold. The Guardian™ Reader is approximately the size of a toaster, and the Defender™ TSR is handheld.

Demonstrated Biodetection Assays

Test strips are available for anthrax, ricin, and botulinum toxins; plague; brucellosis; and pox. Alexeter reports the LOD for anthrax spores to be 1 to 5 million CFU/mL or ~225,000 to 1.1 million spores applied to the test strip (assuming a 3 drop/150 µL sample). A weblink to Alexeter-reported performance specifications is provided in References. A previous evaluation performed by the U.S. EPA Technology Verification (ETV) Program in 2004 does not apply to the current version of



The Guardian™ Reader™



A BioDetect™ Test Strip for anthrax



The Defender™ Reader

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes

Assay time: ~15 minutes

Detection limit (reported by manufacturer):

1 to 5 million CFU/mL (anthrax spores); 5 to 10 ng/mL ricin

Required sample preparation? Minimal

Automatic results display? Operator or instrument reads results

Unit weight: (Optional readers)

Guardian™: 4 lb; Defender™: 1.5 lb

Power: (Optional readers) Rechargeable battery (25 test/charge) or AC

System cost: Strips: \$685 (box of 25), Guardian™: \$6500; Defender™: \$9995

Additional costs: Factory calibration is required every 5 years for either reader (\$750)

Shelf-life: 18 months (ricin assay); 24 months (all others).

the test strips, which include different reagents.

References

No publicly available, peer-reviewed references were found for this product.

Alexeter Anthrax (*B. anthracis*) Bio-Detect Test™ Specifications. Summary provided by Alexeter.
http://www.alexeter.com/biow/products/products/strips/spec%20sheets/Anthrax%20Test%20Strip%20Specifications%20Bio-Detect%20_1p_.pdf

Alexeter: RAID™ Multi-Test Strips

Website: <http://www.alexeter.com/biow/index.asp>

Contact: Tom Fryzel (tfryzel@alexeter.com)

Phone: (877) 591-5571

Technology Summary

Alexeter RAID™ 5 and RAID™ 8 are multiplex LFA strips, which can test simultaneously for five and eight different targets, respectively, from a single sample. Each cartridge comes with a sampling kit that includes an integrated swab, buffer, and dropper dispenser. In the RAID tests, 6 drops of sample (~300 µL) are applied to each sample window (RAID™ 5 has a single window; RAID™ 8 has two windows). These multiplexed cartridges work on the same principle as the single target BioDetect™ test strips: if a biothreat is present, a colorimetric reaction occurs and a line appears in the “T” zone of the results window. For valid results, a line must be observed in the “C” zone (internal positive control) of the results window. These assays are not compatible with the Alexeter Defender™ TSR or Guardian™ Reader.

Demonstrated Biodetection Assays

The RAID™ 5 has assays for *B. anthracis*, *Y. pestis*, and botulinum, ricin, and SEB. The RAID™ 8 adds three additional assays: *Brucella*, orthopox, and *Francisella*. Alexeter reports the following detection limits: anthrax 100,000 CFU/ml (this equates to approximately 30,000 spores, assuming a 300 µL sample is applied), botulinum toxin 30 ng/ml, brucellosis 1.5 million CFU/ml, orthopox 1.6 million PFU/ml, plague 36,000 CFU/ml, ricin 6 ng/ml, SEB 10 ng/ml, and tularemia 1.6 million CFU/ml. A summary of performance specifications can be requested from Alexeter (see References for details).

References

No publicly available, peer-reviewed references were found for this product.

Alexeter RAID 8 BioThreat Detection Kit™ Specifications. Summary of performance specifications available by request from Alexeter.



RAID 8

A multiplex, eight-target immunoassay cartridge from Alexeter

Performance Summary

Anthrax detection? Yes
Ricin detection? Yes
Assay time: ~15 minutes
Detection limit (reported by manufacturer):
 100,000 anthrax spores/mL; 6 ng/mL Ricin
Required sample preparation? Minimal
Automatic results display? Operator reads/interprets results
Unit weight: negligible
Power: N/A
System cost: RAID™ 5: \$695/box of 10 (\$69.50 each), RAID™ 8: \$995/box of 10 (\$99.50 each)
Additional costs: N/A
Shelf-life: 18 months

Environics: ENVI Assay System

Website: www.environicsusa.com

Contact: Chris Wrenn (sales@environicsusa.com)

Phone: (410) 612-1250

Technology Summary

Environics markets the ENVI assay, a colorimetric LFA. The ENVI assay can be read by eye or with a reader. The Envionics Reader Module is designed to be an add-on module for the ChemPro100 Chemical Detector, but also can be used alone, connected to a laptop computer. The reader can be powered by a USB port or directly from the ChemPro100 Chemical Detector. Colorimetric ENVI assays are available for SEB, ricin, and botulinum toxins. Each assay includes all materials required for analysis (e.g., sampling swabs, buffers, transfer pipettes) packaged in a box that doubles as a sample-preparation platform.

Demonstrated Biodefense Assays

Colorimetric ENVI assays are available for the detection of ricin, SEB, and botulinum toxin with reported sensitivities of ricin 5 ng/mL, SEB 13.1 ng/mL, and botulinum toxin 10 ng/mL.

References

No publicly available, peer-reviewed references were found for this product; however, test reports may be requested from the vendor.



The ENVI Assay System: ChemPro100 Chemical Detector, Reader Module, and immunoassay kit

Performance Summary

Anthrax detection? Currently not available

Ricin detection? Yes

Assay time: ~15 minutes

Detection limit (reported by manufacturer):

5 ng/mL ricin; 10 ng/mL botulinum toxin

Required sample preparation? Minimal

Automatic results display? ENVI assays can be read/interpreted by the operator or read using the ChemPro Detector and reader module

Unit weight: ChemPro100+reader: 2.3 lb

Power: ChemPro100+reader: rechargeable Li-ion batteries (8 hr)

System cost: Assays: \$400/box of ten or \$50/each; ChemPro100: \$14,995;

Reader Module: \$3452

Additional costs: None

Shelf-life: 1 to 2 years

MSA/QTL: BIOSENSOR™ 2200R

Website: <http://www.msafire.com/> (sales and info) or <http://www.qtlbio.com> (info)

Contact: Norm Davis (Norm.Davis@MSAnet.com) or Brian Oswalt (boswalt@qtlbio.com)

Phone: (724) 776-8884 (MSA) or (724) 575-0033 (QTL)

Technology Summary

The BIOSENSOR™ 2200R (developed by QTL Biosystems and sold in the United States by MSA) is a fluorescence-based immunoassay developed for downrange biothreat detection. A red light/green light (threat present/absent) result is delivered in 5 minutes. The assay protocols are set up for powder samples, but wet samples may also be evaluated. Each disposable assay cartridge kit comes with a sample collection swab, a sample vial, and syringes for sample and wash buffer delivery into the cartridge. Once a sample and wash buffer are injected into the cartridge, the user shakes the cartridge manually for 1 minute to dissolve the sensing materials (i.e., a pair of antibodies that both bind to the target)—one has a fluorescence label for detection the other has a magnetic bead attached for immobilization on the sensor. A complex is formed between the antibodies and target biothreat molecules in solution, and then a magnetic field is applied to concentrate the magnetic beads and complexes into a pellet on the sensing surface. After the pellet is formed, the operator is prompted to depress the wash syringe plunger to remove any potentially interfering materials before the fluorescence is read. Protocols for sample processing are laminated inside the lid of the Pelican™ case where the unit is housed and the instrument prompts the operator at each step in the process. To verify a positive result, the sample must also be analyzed using a negative control cartridge. Likewise, to verify a negative result, a positive control cartridge must also be analyzed. MSA has a comprehensive training video on YouTube (BIOSENSOR™ 2200R.mp4) that demonstrates the process with an operator wearing Level A protection.

An updated version of this system, the BIOSENSOR 4000, is in development by QTL. This system automates the mixing and washing processes, which improves the ease-of-use, precision, and sensitivity of the system, and updates the optics, allowing additional multiplexing capabilities. The manufacturer estimates that the BIOSENSOR 4000 system will be priced below \$20,000.



The BIOSENSOR™ 2200R and assay cartridge

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes

Assay time: ~5 minutes

Detection limit (reported by manufacturer):

<10,000 spores; < 1 ng ricin

Required sample preparation? Minimal

Automatic results display? Yes

Unit Weight: 7 lb

Power: Battery (50 tests) or AC

System cost: Assays: \$80 (single target tests); \$100 (ricin/anthrax dual test); \$70 negative control cartridge; \$70 positive control cartridge; \$1600 low volume cartridge plan; Reader: \$16,450 (starter kit includes cartridge starter kit, battery charger, carrying case, instruction sheet and manual)

Additional costs: \$3750 to 11,200 and 1 to 4 year extended warranty (optional)

Shelf-life: 16 months

Demonstrated Biodefense Assays

Assay cartridges are available for anthrax and ricin (both as single tests and a duplex). Third-party testing of the anthrax assay, conducted by the University of Alabama (UAB) and the New York State Department of Health (Wadsworth Center), found the LOD to be between 5000 (NYDOH) and 12,000 (UAB) spores depending on the strain and spore preparation method. Weblinks to test reports are provided in References. In 2006, the EPA ETV program evaluated the Biosensor™ 2200R and reported the LOD for anthrax spores to be between 100,000 and 500,000 spores/mL and ricin to be between 0.05 and 0.25 mg/L (weblink to report provided in References). In both assays, interference was observed in samples containing magnesium and calcium; however, the manufacturer reports the buffer has been reformulated since this testing and the assay is no longer affected by these metal ions.

In addition, MSA sells cartridge quantities in plans designed for low (1 call/2 months), medium (2 calls/month), and high volume (5 calls/month) usage. As an example, the low volume plan includes: six anthrax/ricin dual kits, two anthrax and two ricin single kits, six positive control cartridges, two negative control cartridges, two calibration kits, and two wet sample kits. Although a calibration kit is available, MSA/QTL maintains it is not necessary and the instrument light sources (LEDs) are stable for 100,000 hours of use.

References

No independently published, peer-reviewed publications were found.

Performance Verification of the QTL Biosensor at the University of Alabama (UAB). Summary of performance testing performed at the University of Alabama. Test performed in September-November, 2004. Study (not peer reviewed) was sponsored by QTL Biosystems.
<http://s7d9.scene7.com/is/content/minesafetyappliances/BIOSENSOR%202200R%20UAB%20Report%20-%20EN>

Evaluation Report on the QTL Biosystems Bacillus anthracis Cartridges and β -unit Reader. Summary of performance testing performed at the Biodefense Laboratory, Wadsworth Center, NY State Department of Health. Study (not peer reviewed) was sponsored by QTL Biosystems.
<http://s7d9.scene7.com/is/content/minesafetyappliances/BIOSENSOR%202200R%20NYDOH%20Report%20-%20EN>

Environmental Technology Verification (ETV) Report for QTL Biosystems, LLC: QTL Biosensor for Detecting Anthrax and Ricin. Testing performed by Battelle between November 2005 and March 2006. <http://www.epa.gov/etv/pubs/600etv06093.pdf>

New Horizons Diagnostics: Smart™-II

Website: http://www.nhdiag.com/profile_one.shtml

Contact: Larry Loomis (larryl@nhdiag.com)

Phone: (443) 543-5755

Technology Summary

The New Horizons Diagnostics (NHD) Smart™-II is a standard LFA. Each device tests for one agent/target at a time. One kit contains the LFA device, plastic droppers, and Chase buffer. A separate collection kit (either an NHD kit or another validated collection system) must be purchased separately. If the sample contains visible debris or large particles, a sample-processing kit should be used (also sold by NHD) prior to loading sample on to the LFA. The test is initiated by adding three drops (~100 µL) of liquid sample to the sample well on the device using the provided dropper. After waiting three minutes for the sample to absorb in the sample well, two drops of Chase buffer is added from the Chase buffer dropper bottle. After a 15-minute incubation, the results can be read. The test produces one of three results: positive—target detected (two lines in the test window, one for the target and one for the internal positive control), negative—target not detected (single line at the positive control zone), and inconclusive—assay error (no lines, or a single line for the target but no control line).

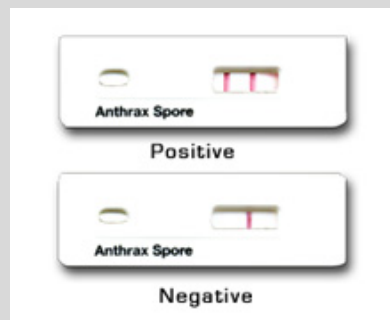
Demonstrated Biodetection Assays

NHD reports the sensitivity for anthrax spores to be 100,000 CFU/mL which translates to 10,000 spores assuming a 3 drop/100 µL sample applied to the device. An independent evaluation sponsored by NHD and performed by the Pacific Northwest National Laboratory (PNNL) supports these findings (report provided upon request from NHD, see References).

References

Performance Assessment of Three Commercial Assays for Direct Detection of *B. anthracis* Spores. Journal of Clinical Microbiology (2003) 3454–3455. This brief study performed by the Florida Department of Health Laboratory, reports detection of 100,000 spores per sample. The volume of the sample applied was not reported.

Task 14: *B. anthracis* lateral flow devices validation. Technical report of PNNL-RCMDT Contract #325786-A-K6. Reporting period: 01.04.05-30.06.05. Report of third-party testing results (not peer reviewed); available upon request from NHD.



Smart™-II lateral flow assay

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes

Assay time: ~15 minutes

Detection limit (reported by manufacturer):
100,000 CFU/mL anthrax spores; 50 ng/mL toxins

Required sample preparation? Minimal

Automatic results display? Operator reads/interprets results

Unit weight: Negligible

Power: N/A

System cost: \$88 (pack of 2);
\$550 (pack of 25)

Additional costs: \$88 Demo/training kit (pack of two); \$23.10 Biosample Collection Kit (five swipes; includes bucket); \$32.89 Biosample Processing Kit (one each); \$322.30 Biosample Processing Kit (ten determinations, includes bucket)

Shelf-life: 12 months

Response Biomedical: RAMP®

Website: www.responsebio.com

Contact: Lindsey Cowan (customersupport@responsebio.com)

Phone: (604) 456-6010 (ext 6076)


Technology Summary

The RAMP® system is a fluorescence-based lateral flow immunoassay system. The assay uses a fluorescence-based detection scheme that requires an optical reader. The complete RAMP® Biodefense System provides all the materials required to run an assay including sampling swabs, buffers, fluorescence reader, and printer in a waterproof Pelican™ case. Four assay kits (anthrax, ricin, botulinum neurotoxin, and smallpox) are provided with the system in addition to a Biodefense Training Kit that is supplied at the free-of-charge onsite training by Emery & Associates. Response Biomedical also provides comprehensive training tutorials for the system on its website. Sample preparation for this assay is slightly more involved than a standard colorimetric LFA; however, the assay is more sensitive than standard LFAs. Initial swab sampling follows standard methods (swab and solubilize in sample buffer). However, transferring the sample to the assay cartridge involves first mixing with the dye-labeled detection antibody, which is dried in the transfer pipette tip. The operator must slowly depress and release the plunger ten times and check that the sample is fully mixed by confirming that the pink dot is no longer visible on the inside of the tip. Once the sample is mixed, the assay proceeds like a typical LFA (add sample to sample window, insert in to the fluorescence reader, and incubate for 15 minutes). Unlike standard LFAs, the positive control that is incorporated into the proprietary RAMP Ratio® corrects for variability in operator technique, sample volume, environmental conditions, and sample viscosity.

As with standard LFAs, RAMP® has an internal positive control, which is automatically read by the system and used for signal processing and quality control when analyzing and displaying the results.

Demonstrated Biodeflection Assays

Response Biomedical has assays available for the detection of anthrax, ricin, botulinum neurotoxin, and smallpox. The anthrax assay is currently the only commercial handheld immunoassay that has a



The RAMP® System

Performance Summary

Anthrax detection? Yes
Ricin detection? Yes
Assay time: ~20 minutes
Detection limit (reported by manufacturer): 4000 spores (400,000 spores/mL), 1 to 100 ng/mL for toxins
Required sample preparation? Fine pipetting and mixing
Automatic results display? Yes
Unit weight: 4.6 lb (20 lb including Pelican™ case and printer)
Power: Battery (100 tests) or AC
System cost: Assays: Anthrax and pox \$675 (box of 25; \$27/test); ricin and botulinum \$545 (box of 25; \$21.80/test); Reader: \$11,333 (starter system includes all the test kits and sample equipment to last a year, a printer, and training)
Additional costs: N/A
Shelf-life: 12 months minimum

“Performance Tested Status” issued from the AOAC Research Institute. The initial performance evaluation was performed in 2004, and the assay has been recertified annually since then as “performing to the manufacturer’s specifications.” In addition, the system was evaluated at ECBC and was found to specifically detect between 1000 and 2000 anthrax spores (weblink to study report provided in References). However, the EPA ETV 2004 evaluation found the assay to be less sensitive, detecting between 7 to 8 million anthrax spores (700 to 800 million spores/mL) depending on spore preparation (weblink to report provided in References). The EPA evaluation did find the assay to be very sensitive to vegetative *Bacillus anthracis* cells (3000 cells or 300,000 CFU/mL).

References

RAMP[®] anthrax test cartridge. *Journal of AOAC International* (2005) 88(1):202-3. This article summarizes the protocol for using the RAMP[®] assay for presumptive laboratory detection of *B. anthracis* spores in environmental samples. No test data are reported.

Method modification (2004.08) to field-testing of visible powders on a variety of nonporous environmental surfaces: field study. *Journal of AOAC International* (2006) 89(6):1622-8. This paper provides a summary of RAMP[®] anthrax assay field-testing performed by trained first responders and civil support teams in Class C personal protective equipment. Test accuracy was at least 97% for all surfaces tested.

Evaluation of the rapid analyte measurement platform (RAMP[®]) for the detection of *Bacillus anthracis* at a crime scene. *Forensic Science International* (2007) 171(1):1-4. This study looked at the accuracy and reliability of the RAMP[®] system for anthrax detection. It found the LOD to be 6200 spores (10 µL sample with a concentration of 620,000 CFU/mL) and no cross-reactivity with closely related near neighbor spores (*Bacillus thuringiensis* and *Bacillus cereus* tested at 100 million spores per 10 µL sample) and no interference observed with samples containing common household powders.

Evaluation of lateral flow assays for the detection of botulinum neurotoxin type A and their application in laboratory diagnosis of botulism. *Diagnostic Microbiology and Infectious Disease* (2007) 57: 243–249. This study reports the detection limit for botulinum neurotoxin type A to be 50 ng/mL using a 10 µL sample diluted into the sample buffer as directed. This study also found the assay detects both purified toxin and toxin in complex with its natural, non-toxic carrier proteins.

Evaluation of a Rapid Immunoassay System for the Detection of *Bacillus anthracis* Spores. Report (not peer reviewed) prepared by US Army Edgewood Chemical and Biological Center, Aberdeen Proving Ground, MD. <http://www.accutest.net/products/pdf/EdgewoodEvaluation.pdf>

Environmental Technology Verification (ETV) Report for Response Biomedical Corp., RAMP Test Cartridges for detecting anthrax, botulinum toxin, and ricin. Testing performed by Battelle between January 14 and April 23, 2004. http://www.epa.gov/etv/pubs/01_vr_ramp.pdf

Research International: RAPTOR™

Website: <http://www.resrchintl.com>

Contact: David McCrae (davidmccrae@resrchintl.com)

Phone: (360) 805-4930/(703) 625-8381

Technology Summary

The RAPTOR™ is a portable assay system first introduced in 2000. It can be configured to detect a wide range of analytes including biological agents, toxins, explosives, and chemicals using antibody-based assays. The RAPTOR™ is essentially a fluorescence reader with integrated fluidics, electronics, and software, which reads proprietary disposable optical waveguide sensors. The system is highly configurable—the user can define multi-step assay protocols and develop their own assays and assay coupons. There are also a range of pre-defined assays and coupons for biothreat targets.

The disposable assay coupons are about the size of a credit card and contain four reaction surfaces (channels containing sensor elements/waveguides) that can be used to simultaneously detect four different target biothreats. The assay coupons can be functionalized by the user, or purchased pre-configured. The pre-configured assay coupons contain a barcode that is automatically read by the instrument and sets up the run parameters for the assay. The system delivers the sample to the reaction surface, which has an immobilized antibody that captures the target on the surface. Then the system can be configured for a wash step (to remove unbound material) before a second fluorescently labeled antibody specific for the target is automatically delivered to the surface. To run an assay, the user adds buffer to the detection antibody (supplied dried in a vial), connects the vial to the system, adds 1 mL of liquid sample to the coupon, inserts the coupon into the reader, and presses the “Run Assay” key. For all assays, the first run performed daily must be a ‘blank’ assay to set the baseline. Each assay including the initial blank run, takes approximately 10 to 15 minutes to complete. If an assay is negative, the coupon can be re-used in an 8 to 12 hour period for up to 30 assays or until a positive result is obtained.



The RAPTOR™ System

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes

Assay time: ~15 minutes

Detection limit (reported by manufacturer):

10,000 spores/mL, 1 to 100 ng/mL for toxins

Required sample preparation? Reconstitute detection antibody dried in vial

Automatic results display? Yes

Unit weight: 12.3 lb, 14.5 lb with battery

Power: Optional military battery BA5590 (9 to 24 hours); rechargeable is also available

System cost: Assays: \$200; Reader module: \$50,000

Additional costs: None

Shelf-life: 6 months

Demonstrated Biodefense Assays

The system is currently used in various settings, including corporate mailrooms and food processing facilities. Assays are available for anthrax, ricin, botulinum toxin, *Y. pestis*, *Francisella tularensis*, and *Vaccinia* virus. Additional assays are available including cocaine and trinitrotoluene (TNT) (explosive). Reported assay sensitivities have been gathered from various third-party research groups and are not guaranteed by Research International. The following LODs have been reported: irradiated anthrax spores

(50,000 spores/mL or 50,000 spores in a 1 mL sample), ricin (1 ng/mL), and botulinum neurotoxin (1 to 10 ng/mL).

References

RAPTOR™: a fluoroimmunoassay-based fiber optic sensor for detection of biological threats. *Sensors Journal, IEEE* (2003) 3(4):352-360. This research paper summarizes the RAPTOR™ design (e.g., optics, fluidics). This paper includes data for research and development assays (not commercial) for detecting SEB and anthrax spores.

Smiths Detection: NIDS™ (Developed by ANP Technologies)

Website: <http://www.smithsdetection.com>

Contact: Ken Klein (ken.klein@smithsdetection.com)

Phone: (302) 353-0909

Technology Summary

Nano Intelligent Detection System (NIDS™) consists of LFA strips and an optical reader for detection. NIDS™ is a standalone component of the Smiths Bio Triage Kit™ that integrates NIDS™ with simple protein/pH tests (BioCheck® 20/20), FT-IR characterization (HazMatID™ 360), and a DNA test (Prime Alert™) for a complete tiered approach using orthogonal assays for evaluating suspicious powders.

NIDS™ immunoassay strips are formatted for the detection of three, four, or five targets in a single cartridge. The manufacturer claims that its proprietary method of immobilizing the capture antibodies in a uniform orientation on the test strips decreases the likelihood of hook effect artifacts that produce false negative results when very concentrated samples are analyzed.

Each test comes with a sampling kit containing transfer dropper bottles, buffer solution, powder scoops, swabs, and sample collection vials. Operation is quite simple, with the user placing 100 µL (about 5 drops) of the sample solution in the sample well of the test strip. The test strip is then allowed to develop for 15 minutes and placed into the palm-sized reader for detection. The results are displayed on the screen.

Demonstrated Biodefense Assays

Multiplexed test strips are available that analyze a single sample for three, four, or five targets simultaneously. Commercial assays are available for the following biothreat targets: anthrax, ricin, plague, botulinum toxin (serotypes A and B), tularemia, smallpox, and SEB. Reported sensitivity for anthrax spores is 7.5 million/mL (or 750,000 spores assuming a 0.1 mL sample is applied to device).

References

No publicly available, peer-reviewed references were found for this product.



The NIDS™ Nano Intelligent Detection System

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes

Assay time: ~15 minutes

Detection limit (reported by manufacturer):
7.5 million spores/mL (anthrax); 12.5 ng/mL (ricin)

Required sample preparation? Minimal

Automatic results display? Yes

Unit weight: 1.6 lb (reader) and 10.1 lb (complete NIDS™ kit)

Power: 3 AA batteries (2 to 3 hours run time)

System cost: Assays: \$45 to 75 (\$15 per target assayed); Reader module: \$7.5K (includes ten pairs of tickets)

Additional costs: None

Shelf-life: 2 years

Tetracore: BioThreat Alert® Test Strips and Reader

Website: <http://www.tetracore.com/bio-warfare/>

Contact: Pete Pillay (ppillay@tetracore.com)

Phone: (260) 268-5400

Technology Summary

Tetracore BioThreat Alert® Test Strips are standard single target/agent LFAs. Each BioThreat Alert® kit (containing 25 strips) comes with 5 sample collection swabs and vials. Additional sample collection kits can be purchased separately. Once a sample is collected and solubilized, the user applies 5 drops (~150 µL) of sample to the test strip sample window. After a 15-minute incubation period, the results can be read by eye in the test window: one line at the control zone indicates the target was not detected, two lines (one control and one test) indicate the target was detected.

For increased accuracy and sensitivity, an optional reader is available. The reader is essentially a tablet-sized, handheld, touchscreen device that provides an objective interpretation of the test results. Following the test incubation period, it takes approximately 30 seconds for the reader to analyze a strip and give an output of positive, negative, or inconclusive. The reader saves and prints the test results, and also includes an attached optional stylus.

Demonstrated Biodetection Assays

Test strips are available for anthrax, abrin, ricin, botulinum toxin, plague, brucella, SEB, tularemia, and orthopox. Tetracore reports the anthrax test to have a sensitivity of 10,000 to 1 million CFU/mL (1500 to 150,000 spores, assuming a 0.15 mL sample volume is applied to the test strip). In 2004, the EPA ETV program evaluated the BioThreat Alert® test strips for the detection of botulinum neurotoxin, anthrax, and ricin (weblink to report listed in the References). Detection limits varied based on the spore preparation (Dugway live spores (700 million spores/mL) vs. Battelle phenol-preserved spores (80 million spores/mL)). Calcium (230 mg/L) and magnesium (90 mg/L) were found to inhibit the anthrax assay; however, calcium and magnesium were not found to interfere with the assays for ricin and botulinum neurotoxin.



The BioThreat Alert® Reader and Test Strips

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes

Assay time: ~15 minutes

Detection limit (reported by manufacturer):

Anthrax: 10,000 to 1 million CFU/mL; ricin: 2 to 5 ng/mL Ricin

Required sample preparation? Minimal

Automatic results display? Automated reader or user observes lines

Unit weight: Reader: 2.1 lb

Power: Not required; optional reader has rechargeable battery (5 hours)

System cost: Assays: \$605/box of 25 (\$24.40/assay); Sample Collection Kit: \$125 (25 sample collections); Reader: \$5500

Additional costs: None

Shelf- life: 2 years for abrin and ricin assays, 3 years for all others .

References

Evaluation of lateral flow assays for the detection of botulinum neurotoxin type A and their application in laboratory diagnosis of botulism. *Diagnostic Microbiology and Infectious Disease* (2007) 57:243–249. This study found the assay did not detect purified botulinum neurotoxin A in the highest concentration samples tested (1,000 ng/mL); however, the toxin in complex with its natural, non-toxic carrier proteins was detected at 10 ng/mL.

Evaluation of Lateral-Flow *Clostridium botulinum* Neurotoxin Detection Kits for Food Analysis. *Applied and Environmental Microbiology* (2005) 71(7):3935–3941. This paper reports the sensitivity for detecting botulinum neurotoxin type A/B to be 10 ng/mL and type E to be 20 ng/mL. This report cites the Alexeter assays, which were originally manufactured by Tetracore for Alexeter but currently, are sold exclusively by Tetracore.

Environmental Technology Verification (ETV) Report for BioThreat Alert® Anthrax, Botulinum Toxin, and Ricin Immunoassay Test Strips. Testing performed by Battelle between January 14 and April 23, 2004. http://www.epa.gov/etv/pubs/01_vr_biothreat.pdf

5.0 PCR-Based Detection Systems

PCR-based assays detect specific organisms based on their DNA sequence. During PCR, protein polymerases copy short pieces of DNA—millions of DNA copies are generated from just a few hundred starting molecules. The assay is designed so only regions of DNA that are unique to the target organism(s) are amplified.

Most field-based PCR systems consist of a disposable assay cartridge containing all of the consumable reagents (including the required polymerase), an instrument that integrates the thermal components required to perform the heat/cool cycles required for PCR, and the optical components required to quantify the amplified DNA products. PCR assays are performed on liquid samples and require a sampling kit to swab a suspicious powder and solubilize the white powder in a buffer compatible with PCR. Sample (white powder + buffer) is added to the assay cartridge and, depending on the system, other manual steps may be required before the cartridge is placed in the instrument. In the instrument, the sample and reaction mixture is cycled between high and low temperatures to amplify the target DNA and the dye-labeled product DNA is measured by integrated optical components (usually fluorescence-based). Each thermal cycle doubles the amount of DNA available for detection; for example, 10 DNA molecules increase to 20, then 40, then 80 in three cycles. An assay typically contains an internal positive control to ensure the system components are performing as specified.

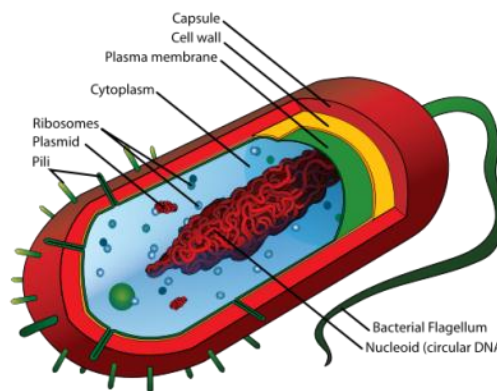
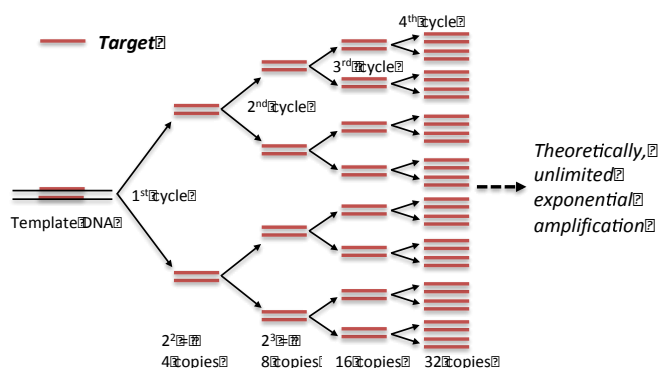


Illustration of a bacterial cell. All cells and viruses contain DNA or RNA that can be used to detect and identify them. (By Mariana Ruiz Villarrea (Average_prokaryote_cell_.svg) [Public domain], via Wikimedia Commons)



PCR can Amplify a Single Piece of DNA to make Millions of Copies in less than 20 Thermal Cycles
 (By Andy Vierstraete (PCRcopies.gif) Yi Cou derivative work (CH391L_S12_PCR_exponential_amplification.jpg) [Public Domain], via OpenWetWare)

PCR-based assays are advantageous because they are very sensitive and specific (although the specificity of a system is dependent on the design of a particular assay). Few field-based PCR systems have integrated sample preparation to concentrate DNA and remove PCR inhibitors; but, because PCR is very sensitive, a sample can often be significantly diluted after sampling to reduce the effects of the inhibitors on the reaction. The most significant disadvantages of PCR-based assays are cost (instruments range from \$15 to 50K and assays from \$12 to 200) and long assay times (30 to 60 minutes). PCR assays will not detect toxins, unless the toxin preparation contains DNA from the source organism (i.e., castor bean DNA in a preparation of ricin toxin).

When presented with several current commercially available hand portable PCR-based systems from Smiths Detection, BioFire Diagnostics (formerly Idaho Technology), and Tetracore, the overwhelming response from the first responders in our recent survey was that none of the systems were user-friendly for a responder wearing a Level A suit. Most of the current systems have preparative steps requiring manual dexterity and visual acuity that would be challenging when the user is fully suited up for a biological threat response. However, most potential biothreat responses are conducted in Level B suits, making some systems easier to use, though still not ideal.

Hand Portable PCR-based Systems

- Assay cost: \$12 to 200
- Reader cost: \$16 to 45K
- Examples: Bio-Seeq™ PLUS (Smiths Detection), T-COR 4™ (Tetracore), RAZOR® EX (BioFire Diagnostics, formerly Idaho Technology), and FilmArray®* (BioFire Diagnostics)

* The FilmArray® system is AC powered, not currently ruggedized, and is more suitable for a mobile laboratory setting

BioFire Diagnostics (formerly Idaho Technology): FilmArray[®]

Website: <http://www.biofiredx.com>

Contact: Lou Banks, Biodefense Marketing Manager (Lou.Banks@biofiredx.com)

Phone: (801) 736-6354

Technology Summary

The FilmArray[®] is a fully automated multiplexed PCR-based platform. The system consists of four components: the loading station, reagent pouch, instrument/reader, and laptop computer (see figure).

After injecting a hydration solution and the sample, all preparation, extraction, amplification, and detection steps are automated. The sample passes through a series of chambers within the reagent pouch. In the first chamber, cells/spores are lysed with ceramic beads and a lysis solution. The next set of chambers purifies and concentrates the nucleic acids using magnetic beads. In the last chamber, RNA is reverse transcribed into cDNA and the first round of multiplexed PCR amplification is performed. The sample is then diluted and partitioned into 120 separate 1 μ L reaction wells, each containing reagents and a target-specific primer pair for a second stage single-plex PCR. In addition, each well contains a fluorescent probe that binds to double-stranded DNA. A patented post-PCR, high-resolution melting point analysis is used to identify positive targets by monitoring the fluorescence quenching of a double-stranded DNA binding probe. The melting curve is dependent on the length, DNA composition and degree of complementarity of the duplex DNA. Software integrates the results from replicate samples and multiple signatures to determine if a sample is reported as positive or negative.



FilmArray[®] System

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes, if castor plant DNA is present

Assay time: ~60 minutes

Detection limit (reported by manufacturer and verified by PNNL): 300 spores/mL

Required sample preparation? Minimal

Automatic results display? Yes

Unit weight: 20 lb

Power: 110V

System cost: \$49,500 (instrument); \$180 per assay pouch (detects 17 biothreat targets)

Additional costs: None

Shelf-life: 6 months

Demonstrated Biodefense Assays

BioFire Diagnostics (BFDx) has a biothreat panel (not currently advertised) pouch that simultaneously tests a single sample for the presence of 27 targets (17 agents): *B. anthracis* (3 targets), Ebola virus, *Variola* virus, *Burkholderia mallei/pseudomallei*, *Francisella tularensis* (2 targets), VEE virus (2 targets), *Rickettsia prowazekii* (2 targets), *Staphylococcus aureus*, *Clostridium botulinum*, Marburg virus (2 targets), *Y. pestis* (2 targets), *Brucella* species (2 targets), *Coxiella burnetii* (2 targets), EEE virus, orthopox (2 targets), *Ricinus communis*, and WEE virus. BFDx reports a sensitivity for anthrax spores to be 300 spores/mL (90 spores, assuming a 300 μ L sample (recommended by BFDx is diluted into the reaction buffer). Initial testing of the FilmArray[®] biothreat pouch by PNNL for detection

of *B. anthracis*, *Francisella tularensis*, and *Y. pestis* genomic DNA samples supports the sensitivity claims of the manufacturer.

References

Evaluation of the Idaho Technology FilmArray[®] system for detection of *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*. *Journal of Applied Microbiology* (first published online January 31, 21013). Evaluation of the FilmArray[®] Biothreat panel with purified DNA samples at PNNL. Sensitivity is reported to be 250 DNA copies.

Evaluation of two multiplex real-time PCR screening capabilities for the detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis* in blood samples generated from murine infection models. *J Med Microbiol.* 2012 Nov;61(Pt 11):1546-55. Evaluation of the FilmArray Biothreat panel for the detection of *Bacillus anthracis* in blood of infected mice. The FilmArray assay was found to have similar detection rates as culture.

The FilmArray[®] system has been FDA-approved for use with the respiratory panel assay cartridge (note: *not* the Biothreat panel) and has been evaluated in three separate studies using clinical samples as reported below. The respiratory panel was both sensitive and selective in detecting several different respiratory viruses in these evaluations.

Comparison of the Luminex xTAG RVP Fast Assay and the Idaho Technology FilmArray[®] RP Assay for Detection of Respiratory Viruses in Pediatric Patients at a Cancer Hospital. *Journal of Clinical Microbiology* 50 (2012) 2282-2288.

Comparison of two broadly multiplexed PCR systems for viral detection in clinical respiratory tract specimens from immunocompromised children. *Journal of Clinical Virology* 53 (2012) 308-13.

Comparison of the Idaho Technology FilmArray[®] system to real-time PCR for detection of respiratory pathogens in children. *Journal of Clinical Microbiology* 50 (2012) 364-71.

BioFire Diagnostics (formerly Idaho Technology): R.A.P.I.D.[®]

Website: <http://www.biofiredx.com>

Contact: Lou Banks, Biodefense Marketing Manager (Lou.Banks@biofiredx.com)

Phone: (801) 736-6354

Technology Summary

Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.[®]) is a portable real-time PCR system. A military version of this system (JB AIDS) is available exclusively to the U.S. Department of Defense. R.A.P.I.D.[®] integrates BioFire Diagnostics' (BFDx) LightCycler[®] instrument technology into a rugged, portable enclosure that fits in a backpack. A laptop computer and sample preparation materials (e.g., vortexer, microfuge, pipettes, racks, wipes) are also included in the backpack. The system requires a 110 or 220V power source for operation.

The system is highly configurable—it can use stock assays and reagents developed by BFDx or independent assays defined by the user. In addition, it is possible to multiplex four signatures in one reaction. Up to 32 samples can be analyzed in parallel in less than 30 minutes, not including sample preparation. Sample preparation is performed offline and can take up to an additional 1.5 hours. Considering the extensive offline sample preparation and external power requirements, this system is well-suited for use in a mobile analytical laboratory or field hospital with trained personnel.

Demonstrated Biodefense Assays

R.A.P.I.D.[®] is compatible with standard real-time PCR assays. All of BFDx's biothreat assays can be run on R.A.P.I.D.[®] (including the 'Biothreat 10 panel'). In a limited evaluation performed in 2004 by the U.S. EPA (weblink to the report provided in the References), the anthrax test was found to detect 2000 CFU/mL consistently with the R.A.P.I.D.[®] *B. anthracis* targets 1, 2, and 3 assays. Assuming a sensitivity of less than 1000 spores/mL, the *B. anthracis* assays can detect 20 organisms, assuming a 20 µL extracted DNA sample is used. Commercial assays are available for the following targets: anthrax, botulinum toxin, *E. coli* 0157, ricin, smallpox, Brucellosis (*Brucella spp.*), tularemia, salmonella, and plague.



The R.A.P.I.D.[®] response kit is essentially a PCR laboratory in a backpack

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes, if castor plant DNA is present

Assay time: ~30 minutes

Detection limit (reported by manufacturer):
<1000 spores/mL

Required sample preparation? Extensive

Automatic results display? Yes

Unit weight: 50 lb

Power: 110V or 220V

System cost: \$55,000 (instrument kit); \$369/box of 40 assays (anthrax target 1 or target 2)

Additional costs: None

Shelf-life: 7 months from manufacture date (however assays may ship with 2 or more months of shelf-life remaining)

References

Idaho Technology Inc. R.A.P.I.D.[®] System for the Detection of *Francisella tularensis*, *Yersinia pestis*, *Bacillus anthracis*, *Brucella suis*, and *Escherichia coli*. EPA Environmental Technology Verification Report 2004. Evaluation of R.A.P.I.D.[®] for detection of potential bioterror agents in drinking water. Sensitivity for *B. anthracis* was found to be 2000 CFU/mL using the Idaho Technology 1-2-3 Flow Kit for sample preparation and R.A.P.I.D.[®] *B. anthracis* targets 1, 2, and 3 assays.

Environmental Technology Verification (ETV) Report for Idaho Technology Inc., R.A.P.I.D. System for the detection of *Francisella tularensis*, *Yersinia pestis*, *Bacillus anthracis*, *Brucella suis*, and *Escherichia coli*. Testing performed by Battelle between May 27 and July 8, 2004.
www.epa.gov/etv/pubs/01_vr_rapid.pdf

BioFire Diagnostics (formerly Idaho Technology): RAZOR® EX

Website: <http://www.biofiredx.com>

Contact: Lou Banks, Biodefense Marketing Manager (Lou.Banks@biofiredx.com)

Phone: (801) 736-6354

Technology Summary

The RAZOR® EX is PCR-based system for pathogen detection in the field. The RAZOR® EX system is packaged in an over-the-shoulder carrying case weighing 11 lb. The system includes a rechargeable internal battery pack that can power the system for eight assays. The system has been extensively tested for field use (e.g., temperature, humidity, vibration, and drop tests) and is currently being employed by a wide range of first responders (e.g., police and fire departments) and friendly governments (e.g., Malaysia, France, Spain, and Germany).

The system performs PCR on a crude sample solubilized from a swab. Typically, a single sample is input into the “reagent pouch,” which contains all the assay reagents (e.g., primers, probes, enzymes, and buffers) needed for the PCR reaction. The system performs minimal sample preparation in the form of sample dilution to reduce the effects of possible PCR inhibitors and thermal disruption of cells and viruses. No additional cleanup of the sample is performed. In preparing the pouch for a run, there are several required manual preparative steps that involve fine manual dexterity. Within the pouch, the sample is split among 12 channels for 12 independent PCR assays. For the BioThreat 10 panel, 10 of the channels are used for pathogen detection (1 signature per channel) with the remaining 2 channels used for control reactions to measure the effect of inhibitors that may be present in a sample. If the sample contains PCR inhibitors, the user is directed to dilute the sample and re-run the assay. Each instrument run takes approximately 30 minutes.



The RAZOR® EX real-time PCR system and single-use reagent pouches

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes, if castor plant DNA present

Assay time: ~30 minutes

Detection limit (reported by manufacturer):
Pathogen dependent (as low as 100 CFU/mL)

Required sample preparation? Minimal

Automatic results display? Yes

Unit weight: 11 lb

Power: battery (8 runs/charge)

System cost: \$38,500 (instrument kit);
\$200/pouch

Additional costs: None

Shelf-life: 6 months

Demonstrated Biodefense Assays

BFDx reports a sensitivity of as low as 100 CFU/mL for anthrax spores, which corresponds to 50 or 200 spores depending on the sample volume (the BA-3 pouch requires 0.5 mL, while the 10-threat pouches require a 2 mL sample). Biothreat assays have been evaluated by end-users in Australia, Canada, and Spain (testing sponsored by BFDx and reported on the BFDx website). The Biothreat 10 target screen kit tests for *B. anthracis*, *Brucella*, *Coxiella*, *E. coli*, *Francisella tularensis*, *Salmonella*, *Y. pestis*, smallpox, ricin toxin, and botulinum toxin. In addition, the RAZOR® platform has been independently evaluated in a Department of Homeland Security-sponsored Stakeholder Panel on Agent Detection

Assays (SPADA) (referenced below) for the analysis of extracts from aerosol collection filters and with manual sample preparation done prior to PCR analysis. This extensive testing found that 2473 of 2479 samples tested provided the expected results (99% success with 95% confidence). Unfortunately, the anthrax assay evaluated in these studies is not commercially available, so the applicability of these results to the currently distributed assay is unknown.

References

Razor[®] EX Anthrax Air Detection System. *Journal of AOAC International* 95(3) (2012) 860-891. This reference summarizes the AOAC Method Developer and Independent Laboratory Validation studies for the RAZOR[®] EX Anthrax Air Detection System. The system could reliably detect 1 pg/reaction volume of *B. anthracis*, which is equivalent to 2000 spores/mL. Unfortunately, these test pouches are not currently commercially available, so the applicability of this study to the commercially available *B. anthracis* assays is unknown.

Smiths Detection: Bio-Seeq™ PLUS

Website: <http://www.smithsdetection.com>

Contact: Ken Klein, Bio-ID Product Manager (ken.klein@smithsdetection.com)

Phone: (203) 207-9700

Technology Summary

The Bio-Seeq™ PLUS is a hand portable PCR instrument that includes a battery for operation in the field. The Bio-Seeq™ PLUS uses cartridges that contain a single target assay and a control assay. Six assay cartridges can be run simultaneously on the instrument. The system relies on thermal stress to disrupt cells/spores during the assay; no specific lysis or DNA sample preparation is performed.

Visual acuity and manual dexterity are required to process samples. After sampling a white powder, a fixed pipettor (provided) is used to pipette 40 µL of sample into the bottom half of a thimble-sized cartridge. The top half of the cartridge is then screwed on the bottom half, and the cartridge is shaken to mix the reagents. The cartridge is then flicked with a snapping wrist motion to move the solution down into the attached cuvette. The cartridge is placed into one of the six ports of the instrument and a run is initiated. Test results are reported as positive, negative, or indeterminate (requires re-reading of the fluorescence signal).

Demonstrated Biodefense Assays

The Bio-Seeq™ PLUS has commercially available cartridges, updated in Spring 2012, that include a new sampling protocol. Commercial assays are available for anthrax (pXO1), anthrax (pXO2), smallpox, tularemia, and plague. The company claims LODs of 3000 to 4000 copies/mL for these assays (120 to 160 anthrax spores assuming a 0.04 mL sample). An evaluation of the previous version of the Bio-Seeq assays, sponsored by Smiths and performed at ECBC, demonstrated the pXO1 assay for anthrax could reliably detect 150 genome equivalents of purified DNA from *B. anthracis* Sterne strain (report available upon request from Smiths, see References). This study also found the pXO2 assay could reliably detect 23,000 genome equivalents for purified DNA from *B. anthracis* Pasteur strain. This detection limit was higher than expected; the loss of sensitivity was attributed to suboptimal reagents provided for testing.



The Bio-Seeq™ PLUS platform consists of a hand portable instrument and dart shaped test cartridges

Performance Summary

Anthrax detection? Yes

Ricin detection? No

Assay time: ~60 minutes

Detection limit (reported by manufacturer; spore detection limit not disclosed): 3750 cells/mL (based on *B. anthracis* vegetative cells)

Required sample preparation? Minimal

Automatic results display? Yes

Unit weight: 6.6 lb

Power: Battery (60 runs/charge)

System cost: \$45,000 (instrument); \$30/cartridge

Additional costs: none

Shelf-life: 18 months

References

Detection of *Francisella tularensis* within Infected Mouse Tissues by Using a Hand-Held PCR Thermocycler. *Journal of Clinical Microbiology* (2003) 41:689-93. This reference describes the detection of *Francisella tularensis* DNA using the original Bio-Seeq™ instrument and reports a detection limit 200 to 300 fg of purified DNA, which is equivalent to approximately 100 to 150 genome equivalents. The same assays were 4 to 6 fold more sensitive when performed on a laboratory-based real-time PCR instrument (ABI 7900).

Evaluation of LATE-PCR Assays for the Identification of Biological Agents using the BioSeeq Plus. Test Analysis Report. (not peer reviewed) Report compiled by US Army Edgewood Chemical Biological Center, Biophysical Chemistry and Biodefense Branches. February 5, 2009. Available upon request from Smiths Detection.

Tetracore: T-COR 4™

Website: <http://www.tetracore.com/t-cor/index.html>

Contact: Pete Pillay (ppillay@tetracore.com)

Phone: (204) 268-5400

Technology Summary

The Tetracore T-COR 4™ is a hand portable, battery-powered PCR instrument. The system is capable of running four independent samples simultaneously. However, only two detection channels are available for each assay; therefore, typically only one target and one internal positive control assay are performed in each assay tube. The instrument kit includes a small, battery-operated centrifuge (pictured) used during the sample preparation.

Sample preparation is not intensive; however, visual acuity and manual dexterity are required. After sampling and solubilizing a suspicious powder with a sampling kit (not provided), 30 µL of sample is transferred into the assay tube. The assay tube is briefly centrifuged to remove bubbles and the tube is then pinched between the thumb and forefingers 8 to 10 times to ensure proper mixing. Finally, the tube is placed into the instrument and the run is initiated.

Demonstrated Biodefense Assays

Tetracore has developed assays to test for a variety of bioagents and the respective diseases they cause, including anthrax, SEB, plague, ricin, tularemia, and botulinum.

References

No publicly available, peer-reviewed references were found for this product.



The T-COR 4™ instrument and battery-powered mini-centrifuge

Performance Summary

Anthrax detection? Yes

Ricin detection? Yes, if castor plant DNA is present in sample

Assay time: ~45 minutes

Detection limit: Not disclosed

Required sample preparation? Minimal

Automatic results display? Yes

Unit weight: 10 lb

Power: Battery (40 runs/charge)

System cost: \$16,000 (instrument);
\$12/cartridge

Additional costs: None

Shelf-life: 12 months



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