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Biodetection Technologies for First Responders: 2015 Edition

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May 2015

http://biodetectionresource.pnnl.gov

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Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table ES.1. Sample Collection Products for Potential Biothreats

Product Name	Manufacturer	Manufacturer Website	Cost	Notes
Alexeter Collection Swab™	Alexeter Technologies, LLC	http://www.alexeter.com	$125/25 pk $5 ea	Sampling swab with integral buffer, mixing chamber, and dropper.
NIDS® Multi-Purpose Sampling Kit	ANP Technologies®, Inc.	http://anptinc.com/	$65/2 pk $32.50 ea	2 collection tubes, 2 scoops, 2 pipettes, 2 buffer containing dropper bottles, and 2 swabs.
SWIPE™-1 Kit: Large Surface Sample Collection Kit	New Horizons Diagnostics, Inc.	http://www.nhdiag.com	$35 ea	Sponge, 25 mL buffer, and slide-lock bag.
SWIPE™-2 Kit: Powder/Small Surface Sample Collection Kit	New Horizons Diagnostics, Inc.	http://www.nhdiag.com	$35 ea	2 swabs, spatula, 25 mL buffer, collection tube, and slide-lock bag.
SWIPE™-3 Kit: Liquid Sample Collection Kit	New Horizons Diagnostics, Inc.	http://www.nhdiag.com	$25 ea	2 syringes and slide-lock bag.
SWIPE™-4 Kit: Air Sampler Sample Collection Kit	New Horizons Diagnostics, Inc.	http://www.nhdiag.com	$30 ea	Collection tube, 25 mL buffer, and slide-lock bag.
SWIPE™ SPK: Sample Processing Kit	New Horizons Diagnostics, Inc.	http://www.nhdiag.com	$50 ea	2 syringes, 2 filters, 2 collection tubes, 25 mL buffer, slide-lock bag, and biohazard bag.
All-in-One Sample Collection Swab	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$30/5 pk $6 ea	Sampling swab with integral buffer, mixing chamber, and dropper.
B2C™ Bulk Sample Collection Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$35 ea	Collection containers, tamper tape, swabs, sample and waste bags, instructions, and chain-of-custody forms.
Biological Sampling Kit (BiSKit™) – Large Area	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$55 ea	Large-area sampling kit.
Chemical, Biological, Radiological, and Explosive (CBRE) Hard Case Sampling Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$3224	Tools for collecting up to 31 total CBRE samples. Ruggedized case.
CBRE Transport Case Sampling Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$3633	Disposable cart. Tools for collecting up to 27 total CBRE samples.
Incident Response Sampling Kit (IRK™)	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$1717	For government only. For sampling air, liquids, or solids.
Mini Push Pack™ Biological Sampling Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$48 ea	Swab, sponge, scalpel, spatula, collection vial, pipette, and other supplies.
Mini Push Pack™ Liquid Sampling Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$110 ea	Syringe, needle, sample container, tubing, and other supplies.

Table ES.1. Sample Collection Products for Potential Biothreats (contd)

Product Name	Manufacturer	Manufacturer Website	Cost	Notes
Mini Push Pack™ Solid Sampling Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$68 ea	Spoon, scalpel, scoopula, sample container, and other supplies.
Mini Push Pack™ Wipe Sampling Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$66 ea	2 alcohol swabs, sample container and bag, extension tool, and other supplies.
QSA Model 102™ Full Forensic Analytical Center (FAC)	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$3260	Large-area sampling kit developed and used by the U.S. Army Mobile Labs and Kits Team.
Residue and Powder Sampling Area Kit (RAPSAK™)	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$435	Powder collection kit for 9 samples. Includes chain-of-custody forms and 2 cameras.
S2P™ Swab Sampling Powder Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$8 ea	Includes swab, buffer, bag, and instructions.
S3™ Bio Sampler and optional S3™ Extension Tool	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$28 ea $36 for tool	Sponge, buffer, pipette, collection tube, and other supplies for surface sampling solid or liquid samples.
Small Area Sampling (SAS™) Kit	QuickSilver Analytics, Inc.	http://www.chembiokits.com	$24 kit $12 ea	Designed for small area sampling. 4-year shelf-life.
BioThreat Alert® Sample Collection Kit	Tetracore, Inc.	http://www.tetracore.com	$125/25 pk $5 ea	3 bottles of buffer, 25 collection vials, alcohol pads and swabs, 3 pairs of tweezers and scissors, 5 scoops, and 1 permanent marker.

Table ES.2. General Biological Indicator Products for Potential Biothreats

Product Name	Manufacturer	Manufacturer Website	Type of Assay	Cost	Notes
Protein or Amino Acid-based					
BioCheck® Powder Screening Kit	20/20 Gene Systems, Inc., 20/20 Bioresponse Division	http://biocheckinfo.com	General biological indicator (protein and pH)	$687/ 25 tests $27.50/test	Colorimetric protein detection and pH test.
Threat Agent Screening Kit (TASKit) BioScreener™	Field Forensics, Inc.	http://www.fieldforensics.com	General biological indicator (protein and starch)	$85/5 tests $17/test	Integral swab and reagents. One colorimetric protein test and one colorimetric starch test.
Pro Hazclass Kit	HazChem, LLC.	http://www.hazchemllc.com	General biological indicator (protein)	$1500 full kit $25/5 tests $5/test	Colorimetric protein detection test.
HazCat Weapons of Mass Destruction (WMD) Kit and 2.0 Pro Kit	HazTech Systems, Inc.	http://www.hazcat.com	General biological indicator (amino acids) and immunoassay	$5285 (WMD Kit) $6100 (2.0 Pro Kit)	All-in-one CBRE detection and classification kits. Also include Alexeter RAID™ 8 immunoassay.
HazCat Anthrax Screening Test Kit	HazTech Systems, Inc.	http://www.hazcat.com	General biological indicator (amino acids)	$805 full kit	All-in-one CBRE detection and classification kit. Also includes Alexeter RAID™ 8 immunoassay.
INDIPRO	Macherey-Nagel, Inc.	http://www.mn-net.com	General biological indicator (protein)	$105/60 tests $1.75/test	Colorimetric protein detection test strip.
ATP-based					
Clean-Trace™ Surface ATP	3M	http://solutions.3m.com	General biological indicator (ATP)	$250/100 tests $2.50/test	Test for live bacterial cells using ATP as an indicator. Optical reader ($2900) required.
Bio-Reveal™	Industrial Hygiene Consulting	http://www.bio-reveal.com/home.html	General biological indicator (ATP)	$250/100 tests $2.50/test	Test for live bacterial cells using ATP as an indicator. Starter kit with optical reader ($1295) required.
PROFILE® 1	New Horizons Diagnostics, Inc.	http://www.nhdiag.com	General biological indicator (ATP)	$450/100 tests $4.50/test	Test for live bacterial cells using ATP as an indicator. Optical reader ($5000) required.

Table ES.2. General Biological Indicator Products for Potential Biothreats (contd)

Product Name	Manufacturer	Manufacturer Website	Type of Assay	Cost	Notes
DNA-based					
Prime Alert®	GenPrime, Inc.	http://www.genprime.com	General biological indicator (DNA)	$70/test	Colorimetric test with a separate immunoassay toxin test. Optical reader ($12,000) required.
FTIR-based					
HazMatID™ 360	Smiths Detection, Inc.	http://www.smithsdetection.com	General biological indicator (infrared protein signature)	$55,000	General biological indicator test for protein using FTIR.
HazMatID™ Elite	Smiths Detection, Inc.	http://www.smithsdetection.com	General biological indicator (infrared protein signature)	$45,000	General biological indicator test for protein using FTIR.
HazMatID Ranger™	Smiths Detection, Inc.	http://www.smithsdetection.com	General biological indicator (infrared protein signature)	$35,000	General biological indicator test for protein using FTIR.
TruDefender™ FT/FTi	Thermo Fisher Scientific, Inc.	http://www.ahurascientific.com	General biological indicator (infrared protein signature)	$45,000 FT $46,500 FTi	General biological indicator test for protein using FTIR.

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

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

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

Product Name	Manufacturer	Manufacturer Website	Cost	Notes
RAPTOR™: Automated, Multianalyte Bioassay Detection System	Research International, Inc.	http://resrchintl.com	$200/assay $50/agent	Automated 4-agent assay (10 assays per box) and detection system ($50,000).
RAMP® assays and optical reader	Response Biomedical Corp.	http://responsebio.com	$24-$27/agent	1-agent assays. 25 assays per box. Required optical reader ($7000).
BioThreat Alert® assays and optical reader	Tetracore, Inc.	http://www.tetracore.com	$24/agent	1-agent assays. 25 assays per box. Optional optical reader ($5500).
iTIRF	TIRF Labs	http://www.tirf-labs.com	$2-$40/assay	Immunoassay- and nucleic acid-based probe total internal reflectance fluorescence biodetector ($9900).
TIRF Sense	TIRF Labs	http://www.i-diagnostics.net	$20-$400/assay	Immunoassay- and nucleic acid-based probe total internal reflectance fluorescence biodetector ($35,000).

Table ES.4. PCR-Based Detection Products for Potential Biothreats

Product Name	Manufacturer	Manufacturer Website	Cost	Notes
FilmArray®	BioFire Diagnostics, Inc.	http://www.biofiredx.com	$185/assay $12/agent ($7/target)	16-agent assay (28 targets) with internal control. 6 assays per box. AC power. Instrument cost = $39,500.
RAZOR® EX	BioFire Diagnostics Inc.	http://www.biofiredx.com	$200/assay $20/agent	10-agent assay with internal control. 1-agent (3-target) assay also available for anthrax ($180). Assays sold individually. Battery power. Instrument cost = $38,500.
One3™	Biomeme	http://www.biomeme.com	$19/agent	1-agent assay with positive and negative control samples. Battery power. Instrument cost = $4950.
POCKIT	Gene Reach USA	http://www.genereach-us.com	$8/agent	1-agent assays. 24 assays per box. No internal control. Battery power. Instrument cost = $8000.
POCKIT MICRO	Gene Reach USA	http://www.genereach-us.com	$12.50/agent	1-agent assays. 24 assays per box. No internal control. Battery power. Instrument cost = $900.
T-COR 8™	Tetracore, Inc.	http://www.tetracore.com	$12/assay $4-$6/agent	2-3 agent assays with internal control. 64 assays per box. Battery power. Instrument cost = $28,500.

Acronyms and Abbreviations

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

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

Contents

Tables

1.0 Biothreat Diseases and Causative Agents

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

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

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

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

Table 1.1. Diseases/Toxins for Category A Priority Pathogens

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

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

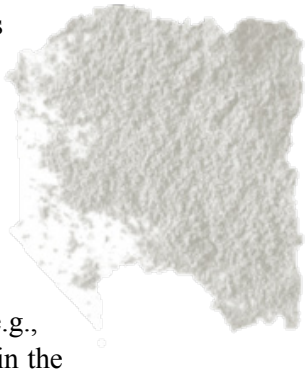
[1] CDC website – http://www.bt.cdc.gov/agent/agentlist-category.asp

Table 1.2. Diseases/Toxins for Category B Priority Pathogens

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

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

2.0 Sample Collection



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

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

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

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

References

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

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

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

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

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

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

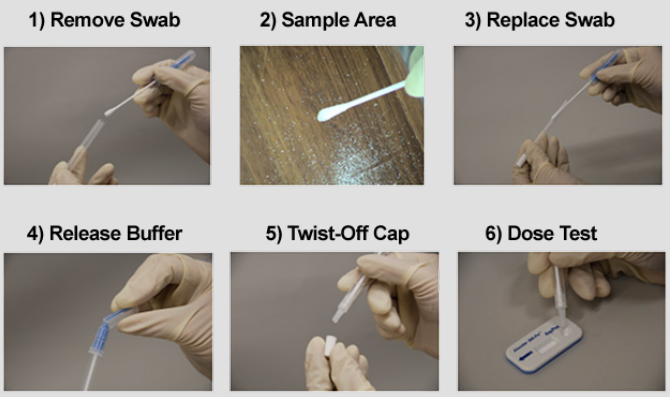
Alexeter Technologies, LLC: Alexeter Collection Swab™

Phone: (877) 591-5571 *Manufacturer's website*: http://www.alexeter.com

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 PBS buffer for sample solubilization, 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. Alexeter also offers a BioDetect™ Standard Collection Kit for use with BioDetect™ immunoassay test strips that contains 25 separate swabs, 5 spatulas, 25 sample vials, 3 bottles of buffer, 100 pH strips, 1 permanent marker, and instructions ($107 [$4.28 ea; picture not available]).

Specifications



Alexeter Collection Swab™

Immunoassay compatible? *Yes.*
PCR compatible? *Yes.*
Submersible decontamination? *Yes.*
Cost: *Swab – $125/25 pack ($5 each).*
Shelf-life: *2 years from date of manufacture.*

Peer-Reviewed References

No peer-reviewed publications were found that evaluate the use of this product for the collection of potential biothreats.

ANP Technologies, Inc.: NIDS® Multi-Purpose Sampling Kit

Phone: (302) 283-1730 *Manufacturer's website*: http://anptinc.com

Technology Summary

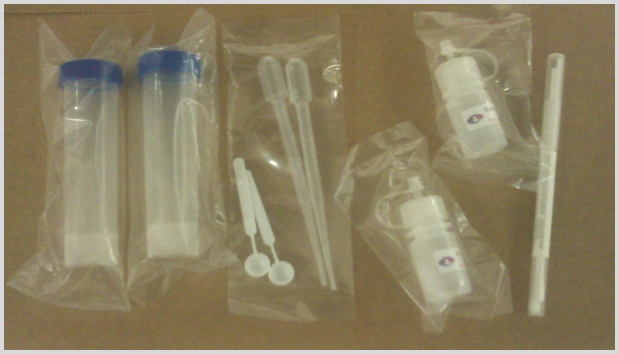
This kit contains supplies for collection of two separate samples. A kit includes two 50 mL free standing collection tubes, two scoops, two transfer pipettes, two 15 mL dropper bottles containing 5 mL of PBST-K buffer, and two swabs.

The management system governing the manufacture of this product is International Organization for Standardization (ISO) 9001:2008-certified (specifies the requirements of a quality management system).

Peer-Reviewed References

No peer-reviewed publications were found that evaluate the use of this product for the collection of potential biothreats.

Specifications



NIDS® Multi-Purpose Sampling Kit

Immunoassay compatible? *Yes.*
PCR compatible? *Unknown.*
Submersible decontamination? *Yes.*
Cost: *Kit – $65/2 pack ($32.50 each).*
Shelf-life: *2 years from date of manufacture.*

New Horizons Diagnostics, Inc.: SWIPE™ Kits

Phone: (443) 543-5755 *Manufacturer's website*: http://www.nhdiag.com/index.htm

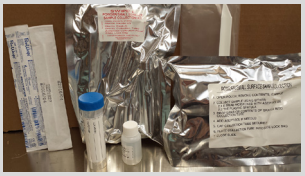
Technology Summary

New Horizons Diagnostics (NHD) 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.

- SWIPE™-1 ($35 each) is a large surface collection kit that includes 25 mL of proprietary buffer, an adenosine triphosphate (ATP)-free sponge, a Whirl-Pak® bag, and a slide-lock bag.
- SWIPE™-2 ($35 each) is a small surface or powder collection kit, which includes a spatula, 25 mL of buffer, collection tube, two swabs, and a slide-lock bag.
- SWIPE™-3 ($25 each) is a liquid collection kit with two syringes, a Whirl-Pak® bag, and a slide-lock bag.
- SWIPE™-4 ($30 each) is an aerosol on a filter collection kit containing a collection tube, 25 mL of buffer, forceps, and a slide-lock bag.

Specifications

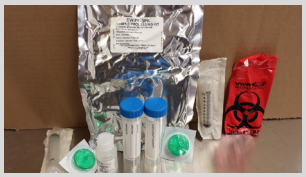
SWIPE™-1



SWIPE™-2

SWIPE™-3

SWIPE™-4



SWIPE™ SPK

Immunoassay compatible? *Yes.*
PCR compatible? *Yes.*
Submersible decontamination? *Yes, includes a slide-lock bag in every kit.*
Cost: *Varies; see text.*
Shelf-life: *3 years from date of manufacture.*

- SWIPE™ Sample Processing Kit (SPK) ($50 each) is a kit designed to be used for sample dilution or pre-filtration of turbid samples and includes syringes, collection tubes, 25 mL of buffer, a biohazard bag for waste collection, and a slide-lock bag.

Peer-Reviewed References

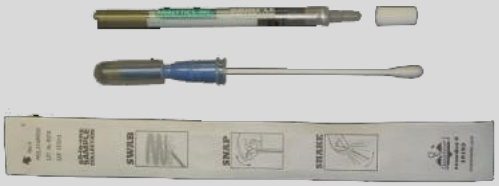
No peer-reviewed publications were found that evaluate the use of any of these products for the collection of potential biothreats.

QuickSilver Analytics, Inc.: Sampling Kits

Phone: (877) 725-7587 *Manufacturer's website*: http://www.chembiokits.com

Technology Summary

This company specializes in sampling kits for the HazMat community. Several biological sampling kits with slightly different utility are described here. All kits use PBS buffer and the sample containers can be dunked for decontamination after sample collection.



All-in-One Sample Collection Swab

B2C™ Bulk Sample Collection Kit

BiSKit™

- All-in-One Sample Collection Swab ($30/5-pack; $6 each). This all-in-one sampler includes buffer, swab, and dropper. After swabbing a surface, the swab is placed back in the collection device, the enclosed buffer compartment is snapped open to release the solution, and the device is shaken to mix the sample and buffer. The integral dropper can then be used to apply solubilized sample to detection assays that require a liquid sample (e.g., PCR or immunoassay).
- B2C™ Bulk Sample Collection Kit ($35). This kit contains sample containers, tamper tape, swabs, laminated cards, sample and biohazard waste bags, field-screening result and chain-of-custody forms, sample collection and instruction sheets, and document pouches. This sampling kit meets the ASTM E2458-10 Method A Standard.
- Biological Sampling Kit (BiSKit™) Large Area Sampling Kit ($55). This kit is designed to sample 1 m^2 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 refastened and the unit can then be removed from the area for later testing or used for immediate analysis. This kit has a 2-year shelf-life from date of manufacture. A reduced-cost training version of this kit—not cleaned or screened/non-sterile components—is also available.

(QuickSilver Sampling Kits, Continued)

- CBRE Hard Case Sampling Kit ($3633). This kit's components are housed in foam-lined compartments in a rugged case. The kit includes tools for sampling for over 10 chemical warfare-related compounds, toxic industrial chemicals (TICS), and toxic industrial materials (TIMS), as well as tools for collecting biological samples. Enough individually packaged supplies are included to collect 9 chemical, 13 biological, 6 radiological, and 3 explosive samples. Individual kit items are available for purchase. The overall weight is 48 lb and the dimensions are 31 in. long x 20 in. wide x 12 in. high. A reduced-cost (13% less) training version of this kit—not cleaned or screened/ non-sterile components—is also available.

- Chemical, Biological, Radiological, and Explosive (CBRE) Transport Case Sampling Kit ($3224). This sampling kit on a three-wheeled cart features a disposable cart to simplify decontamination efforts. It includes tools for sampling for over 10 chemical warfare-related compounds, TICS, TIMS, and tools for collecting biological samples. Enough individually packaged supplies are included to collect nine chemical, nine biological, six radiological, and three explosive samples. Individual kit items are available for purchase. The overall weight of this sampling kit is 38 lb, and the dimensions are 18 in. long x 11 in. wide x 25 in. high. A reduced-cost (25% less) training version of this kit—not cleaned or screened/non-sterile components—is also available.

- Incident Response Sampling Kit (IRK™) ($1717). This kit is designed for use by emergency responders and contains all materials necessary to collect air, liquid, and solid samples for chemical and biological analysis. Instructions and flow-charts are included that detail preparation for entry into the hot zone and sample transfer to law enforcement. The kit meets U.S. Environmental Protection Agency cleanliness standards. Individual kit items are available for purchase. The overall weight is 40 lb and the dimensions are 18 in. wide x 12 in. high x 24 in. diameter.

CBRE Hard Case Sampling Kit

CBRE Transport Case Sampling Kit

Incident Response Sampling Kit (IRK™)

(QuickSilver Sampling Kits, Continued)

- Mini Push Pack™ Sampling Kits. These kits are highly compact and portable single-use units that contain all the tools and supplies (i.e., sample bags, spatulas, scalpels, swabs, pipettes, vials, and Parafilm®) needed to take a single sample. Kits are available for a variety of samples (see below). Each type of kit is identified by a color-coded strap. Following the sampling procedure, the kit can also be used for waste disposal by placing all waste on the absorbent liner, rolling it up, and securing with the Velcro® strap. Training versions (containing uncleaned or screened/non-sterile components) of the kits are available for a discounted price (40 to 55% less). Mini Push Pack™ Sampling kits include the following: Biological Sampling Kit ($48), Liquid Sampling Kit ($110), Solid Sampling Kit ($68), and Wipe Sampling Kit ($66).

Mini Push Pack™ Kits:

Bio Sampling Kit

Liquid Sampling Kit

Solid Sampling Kit

Wipe Sampling Kit

- QSA Model 102™ Full Forensic Analytical Center (FAC) ($3260). This is a comprehensive sampling kit contained in a backpack for chemical, radiological, and biological agent sampling. This kit was initially developed by the Edgewood Chemical and Biological Center FAC Mobile Labs Team, and the kit includes enough single packaged supplies to collect nine chemical, nine biological, six radiological, and three explosive samples. Individual kit items are available for purchase. The overall weight is 32 lb and the dimensions are 22 in. wide x 13 in. high x 11 in. diameter. A reduced-cost (22% less) training version of this kit—not cleaned or screened/non-sterile components—is also available.

QSA Model 102™ Full FAC

Residue and Powder Sampling Area Kit (RAPSAK™)

- Residue and Powder Sampling Area Kit (RAPSAK™) ($435). This lightweight toolbox sized kit is designed for collecting residue and powder samples. The kit includes single-use tools (for collecting up to nine samples of different sizes), BiSKit™ and SAS™ sampling kits, chain-of-custody forms, a disposable camera, tamper seals, pens, markers, and a ruler. The overall weight is 12 lb and the dimensions are 19 in. long x 10 in. high x 10 in. wide.

(QuickSilver Sampling Kits, Continued)

- S2P™ Swab Sampling Powder Kit ($8). This kit contains a swab, buffer solution, a sample bag, a sample collection sheet, and an instruction sheet. The sampling kit meets the ASTM E2458-10 Method B Standard.
- S3™ Bio Sampler ($28). This device is designed to collect biological samples from potentially contaminated surfaces onto a PBS buffer-moistened sterilized sponge for field or laboratory analysis. The S3™ Extension Tool ($36), for sampling hard to reach locations, is not shown.
- Small Area Sampling (SAS™) Kit ($24/kit; $12 each). This kit is intended to sample small areas and includes two swabs that can be moistened with the provided buffer (4 mL of PBS) for sampling powders or used directly for liquids. The swab can be broken off into the buffer bottle and placed into the 50 mL centrifuge tube and provided plastic bag 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.

The management system governing the manufacture of these products is ISO 9001:2008-certified (specifies the requirements of a quality management system).

S2P™ Swab Sampling Powder Kit

S3™ Bio Sampler

SASTM Kit

Specifications

Immunoassay compatible? *Varies; see text.*
PCR compatible? *Varies; see text.*
Submersible decontamination? *Varies; see text.*
Cost: *Varies; see text.*
Shelf-life: *Varies; see text.*

Peer-Reviewed References

Buttner, M. P.; Cruz, P.; Stetzenbach, L. D.; Klima-Comba, A. K.; Stevens, V. L.; Emanuel, P. A. Evaluation of the Biological Sampling Kit (BiSKit) for Large-Area Surface Sampling. *Appl. Environ. Microbiol*. **2004**, *70,* 7040-7045. DOI: 10.1128/AEM.70.12.7040-7045.2004.

The BiSKit™ was evaluated and compared to two other sampling approaches. *Bacillus atrophaeus* spores were used as a simulant for *Bacillus anthracis* and deposited from an aerosol onto wood laminate and metal surfaces. Manufacturer guidance regarding the sampling area was followed for BiSKit (1 m^2), Critical Reagents Program cotton swab sampling kit (100 cm^2 sampling area), and ASD's foam swab sample processing kit (317 cm^2). Culturing, quantitative PCR, and immunoassays were used for analysis, and the results were compared based on the total number of spores collected and spores collected per unit area sampled. The BiSKit collected fewer spores per unit area than the swabs, but collected more spores per sample due to the larger area sampled. In general, spore recovery was greater from metal surfaces than from wood laminate surfaces. The BiSKit was evaluated for collecting both wet and dry samples. It was noted that delaying suspension of a sample improved its stability for later analysis.

Tetracore, Inc.: BioThreat Alert® Sample Collection Kit

Phone: (240) 268-5400 *Manufacturer's website*: http://www.tetracore.com/index.html

Technology Summary

The BioThreat Alert® sampling kit is designed for the collection of up to 25 samples and includes 25 swabs, 25 sample vials, 25 alcohol pads, 3 bottles of sample buffer (12 mL each), 5 scoops, 3 scissors, 3 tweezers, 1 permanent marker, and instructions for use.

Peer-Reviewed References

No peer-reviewed publications were found that evaluate the use of this product for the collection of potential biothreats.

Specifications

BioThreat Alert® Sample Collection Kit

Immunoassay compatible? *Yes.*
PCR compatible? *No.*
Submersible decontamination? *No.*
Cost: *Kit – $125 ($5 each).*
Shelf-life: *1 year from date of manufacture.*

3.0 General Biological Indicator Tests

The purpose of a biological indicator technology is simply to detect the potential presence of biological material in a sample. Typically, these tests detect proteins, amino acids, deoxyribonucleic acid (DNA)/ribonucleic acid (RNA), or adenosine triphosphate (ATP).

Proteins, amino acids and DNA are found in all cells, including skin cells, spores, and bacterial cells. ATP is a metabolite found only in living cells. Biological toxins (e.g., ricin and botulinum toxins) are typically protein-based, but not always. Toxin samples may contain DNA if the material is crudely prepared from the cells that produced the toxin. For example, the potential presence of botulinum or ricin toxin is indicated by the presence of DNA from *Clostridium botulinum* and *Ricinus communis*, respectively.

Fourier Transform infrared (FTIR) spectroscopy is a common analytical tool used by first responders for screening suspicious samples that can help identify a chemical substance. It may also be useful for indicating the potential presence of biological material. FTIR provides information about sample composition by matching the spectral fingerprint of the sample to a library containing spectral fingerprints for thousands of compounds. FTIR is primarily used as a screening tool. If a sample's spectrum is not in the library, the instrument software algorithm will attempt to identify it based on chemicals that are in the library and that have similar spectral features. A "score" is assigned and displayed to the user for potentially matching chemicals in the library, and typically ranges from 0 (no match) to 1 (a perfect match). The score does not represent relative concentration and does not represent probability of correct identification. However, scores >0.9 often, but not always, indicate that the sample is very similar to the substance indicated by the library match. Some FTIR spectrometers include spectral analysis algorithms to indicate that a sample may be of biological origin based on the presence of protein, but FTIR has not been extensively tested for detection of biological material in powders.

General biological screening tests detect a broad range of biological and organic materials, but do not confirm the presence of a specific biothreat agent. Therefore, while many of the biological indicator tests are relatively rapid and inexpensive, they should be used as a screening tool in conjunction with more specific tests. In general, biological indicator tests have low specificity (i.e., not unusual for a false-positive result) and many have low sensitivity (i.e., may result in a false-negative result).

Protein or Amino Acid Test

- Detects any type of protein or amino acid, which are present in all cells (e.g., anthrax, human cells, and non-hazardous bacteria), but are also present in common consumer products (e.g., coffee creamer and powdered infant formula).
- Protein tests may also include a pH test (biological material is typically, but not always, neutral in pH) or a starch test (indicative of a food ingredient, but does not exclude the potential presence of a biothreat)
- Easy to use (add or swab sample, mix, and visually read)
- Operator manually reads color change for protein, amino acids, pH, or starch
- Sample to answer in 5 minutes or less
- LOD: ~100 million *Bacillus anthracis* spores (equivalent to about 10,000 infectious doses, which is barely a visible amount of powder, ~0.1 mg)

- Typical assay cost: assays come packaged in various quantities and costs range from $2 to $28 each
- Typical shelf-life: 14-24 months
- Examples:
  - BioCheck® (20/20 Gene Systems)
  - TASKit BioScreener™ (Field Forensics)
  - HazClass® Kits (HazChem®)
  - HazCat® Kits (HazTech Systems)
  - INDIPRO (Macherey-Nagel)

ATP Test

- Tests if any type of cellular material is present and alive
- Moderate ease-of-use (involves several steps including pipetting and filtering steps)
- Note: for spores an additional spore germination step (~15 minutes) must be performed prior to detection to stimulate the spore to convert to a vegetative (live cellular) state to enable detection. Not all of these products include reagents for spore germination.
- Requires an optical reader
- Sample to answer in about 20 minutes
- LOD: ~1 million *Bacillus anthracis* spores if an additional spore to vegetative cell conversion step is performed (equivalent to about 100 infectious doses, which is a nearly invisible amount of powder, ~0.001 mg)
- Instrument cost (optical reader): $1200 to $5000
- Typical assay cost: ~$3 to $5
- Shelf-life: ~12 months (temperature dependent; some reagents require refrigeration)
- Examples:
  - Clean-Trace™ (3M)
  - Bio-reveal™ (Industrial Hygiene Consulting)
  - PROFILE® 1 (New Horizons Diagnostics)

DNA Test

- Detects any type of DNA (e.g., human, plant, or animal) and some types of RNA
- Easy to use (add sample, mix, and read)
- Requires a fluorescence optical reader
- Sample to answer in about 5 minutes
- LOD: ~10 million *Bacillus anthracis* spores (equivalent to about 1000 infectious doses, which is just a residual, barely visible, amount of powder, ~0.01 mg)
- Instrument cost (fluorometer): $12,000

- Typical assay cost: $70
- Typical shelf-life: 12 months

Example: Prime Alert® (GenPrime®)**FTIR Spectroscopy**

- Primarily used to rapidly identify chemical composition of an unknown sample
- Proteins (contained in most biological materials) give a unique FTIR spectrum and *should* be detected in samples if they have a protein content of at least 10%
- FTIR has not been extensively tested as a screening tool for biological material in suspicious powders
- Identification of sample composition is based on comparison of the sample's composite spectrum to a library of known individual component spectra
- Moderate ease-of-use
- Mixtures can be difficult for software algorithms to correctly identify individual substances
- Sample to answer in <5 minutes
- LOD: ~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 such as additional library spectra ($35,000 to $55,000)
- No consumables (no per test cost)
- Examples:
  - HazMatID™ 360 (Smiths Detection)
  - HazMatID™ Elite (Smiths Detection)
  - HazMatID Ranger™ (Smiths Detection)
  - TruDefender™ (Thermo Scientific)

In the remainder of this section we have grouped together the general biological indicator tests based on technology type: protein and amino acid tests, ATP Tests, DNA tests, fluorescence particle detection, and FTIR spectroscopy.

20/20 Gene Systems, Inc.: BioCheck® Powder Screening Kit (Protein Test + pH Test)

Phone: (240) 453-6339 (Ext. 103) *Manufacturer's website*: http://biocheckinfo.com

Technology Summary

The BioCheck® Powder Screening Kit is a tool to screen for biological material in visible powder samples. This test is not designed for environmental sampling (e.g., soil). A sample is collected with the provided swab, added to a liquid-containing tube, and allowed to incubate for 5 minutes. If the sample contains sufficient protein, the solution in the tube turns purple. 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 will turn the solution purple. A positive protein result is a tentative indication of the presence of biological material. The pH test will turn pink/red if the sample is acidic, blue 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 if a biological agent of concern may be present or if the sample merely contains a harmless protein-containing substance (e.g., brewer's yeast or powdered milk). This kit is also distributed by numerous other companies including Alexeter Technologies, Fisher Scientific, Haz Tech Systems, Laurus Systems, Safety Solutions, and Smiths Detection.

Specifications

BioCheck® Powder Screening Kit for Detection of Protein and pH

General Biological Indicator Tests		
Assay	**Biological Indicator**	**LOD***
BioCheck®	Protein	12 µg**
BioCheck®	pH	Not applicable
*Reported in peer-reviewed reference **Approximately 10 million spores or 12 µg of ricin toxin		

Assay time: *~5 minutes.*
Basis of Detection: *Presence of protein; does not distinguish between biothreat and non-threat materials.*
Required sample preparation? *No.*
Automatic results display? *User interprets color change.*
Unit weight: *Negligible.*
Power: *None.*
Cost: *Assay – $687/25 pack ($27.50 each).*
Additional costs: *None.*
Assay shelf-life: *12 months from date of manufacture.*

This product has received a "Designated" classification (proven effectiveness, with confidence of repeatability) by DHS as part of its Support Anti-terrorism by Fostering Effective Technologies (SAFETY) Act of 2002 (www.safetyact.gov).

Peer-Reviewed References

Poore, C.; Clark, P.; Emanuel, P. A. An Evaluation of Suspicious Powder Screening Tools for First Responders. *J. Hazard. Mater.* **2009**, *172*, 559-65. DOI: 10.1016/j.jhazmat.2009.05.142.

This study compared the effectiveness of three general biological indicator tests to differentiate suspicious (but harmless) powders from biological threat agents. Test samples included *Bacillus anthracis* (Sterne strain) (washed 2 or 4 times to reduce extraneous DNA and cellular protein on spore surfaces),

Yersinia pestis (A1122 strain), and purified ricin. These studies were performed with biothreat samples added directly to the kit solutions; thus, LODs are not reported as substance per milliliter (i.e., concentration). The 20/20 Gene Systems BioCheck® Powder Screening kit (protein test) could detect 1×10^8 colony-forming units (CFU) of *Bacillus anthracis* (4X wash), 1×10^7 CFU of *Bacillus anthracis* (2X wash), 1×10^7 CFU of *Yersinia pestis*, and 100 µg of ricin. The GenPrime Prime Alert® (DNA test) could detect 2×10^{10} CFU of *Bacillus anthracis* (4X wash), 1×10^9 CFU of *Bacillus anthracis* (2X wash), and 1×10^8 CFU of *Yersinia pestis.* The New Horizons Diagnostics PROFILE® 1 (ATP test) could detect 1×10^4 CFU of *Bacillus anthracis* (both 2X and 4X wash) and 1×10^6 CFU of *Yersinia pestis.* Because purified ricin was used, the Prime Alert® and PROFILE® 1 were unable to detect ricin as neither DNA nor ATP were present. Powders were also tested after spiking with biological agents. In general, all kits gave positive results for powders that contained the targeted substance (e.g., protein or cells), but some powders caused interferences and impacted the sensitivity as discussed in the Conclusions section of the paper.

Field Forensics, Inc.: TASKit BioScreener™ (Protein Test + Starch Test)

Phone: (727) 490-3609 (Ext. 105) *Manufacturer's website*: http://www.fieldforensics.com

Technology Summary

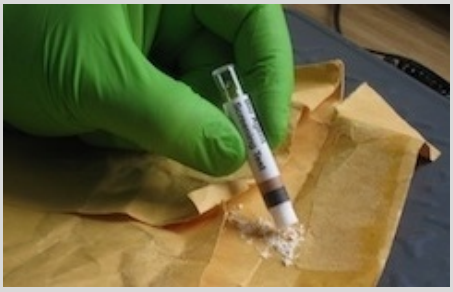
The Threat Agent Screening Kit (TASKit) BioScreener™ includes two easy-to-use tests for rapidly screening suspicious powders for the presence of biological agents. The first colorimetric test indicates if protein is present. A positive protein result suggests a biological agent or toxin *may be* present in the sample. If the protein test is positive, additional tests should be performed to determine if a biological agent of concern is actually present or if the sample merely contains a harmless protein-containing substance. The second colorimetric test indicates if starch is present, which may indicate that the sample is a harmless substance (e.g., a food product). However, additional confirmatory tests are needed to determine if a biological agent is present.

The BioScreener™ test is primarily marketed for homeland security applications and first responders. Both the protein and starch tests can be completed in <1 minute. The swab and reagents are conveniently packaged in a single disposable tube.

Peer-Reviewed References

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

Specifications



TASKit™ BioScreener Tests for Detection of Protein and Starch

General Biological Indicator Tests		
Assay	**Biological Indicator**	**LOD**
BioScreener™	Protein	Not reported
BioScreener™	Starch	Not reported

Basis of Detection: *Presence of protein; does not distinguish between biothreat and non-threat materials. Starch test assists with possible identification of a possible food product.*
Assay Time: *<1 minute.*
Basis of detection: *Presence of protein; does not distinguish between biothreat and non-threat materials.*
Required sample preparation? *No.*
Automatic results display? *User interprets color change.*
Unit weight: *Negligible.*
Power: *None.*
Cost: *Assay – $85/5 pack ($17 each).*
Additional costs: *None.*
Assay shelf-life: *24 months from date of manufacture (-40-65°C).*

HazChem®, LLC: Pro HazClass® Kit

Phone: (303) 259-1501 *Manufacturer's website*: http://www.hazchemllc.com/

Technology Summary

The HazChem® Pro HazClass® Kit is marketed to first responders for detecting protein in suspicous powder substances. Although this kit does not provide identification of the biological agent, the presence or absence of protein in the sample can be determined. If the protein test is positive, additional tests should be performed to determine if a biological agent of concern is actually present or if the sample merely contains a harmless protein-containing substance.

Testing is performed by collecting and placing the sample in a spot dish and directly depositing a test reagent onto the sample. If the concentration of protein in the mixture (test sample and reagent) is 1% or greater, a color change from blue to purple is observed.

Peer-Reviewed References

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

Specifications

Pro HazClass® Kit for Detection of Protein

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
Anthrax	Protein	1%**

*Reported by manufacturer.
**~100 million spores in a 10 mg sample

Assay time: *~5 minutes.*
Basis of Detection: *Presence of protein; does not distinguish between biothreat and non-threat materials.*
Required sample preparation? *Minimal.*
Automatic results display? *User interprets color change.*
Unit weight: *Negligible.*
Power: *None.*
Cost: *Assay – $25/5 tests ($5 each). $1500 initial cost for the full kit.*
Additional costs: *None.*
Assay shelf-life: *24 months from date of manufacture.*

HazTech Systems, Inc.: KT7001 HazCat® WMD Kit and KT7003 HazCat® 2.0 Pro Kit (Protein Tests + Various Assays)

Phone: (800) 543-5487 *Manufacturer's website*: http://www.hazcat.com

Technology Summary

The KT7001 HazCat® Weapon of Mass Destruction (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 KT7003 also includes the capabilities to test for nuclear, chemical, narcotics, and biological agents.

Both kits include biological agent assays for detecting amino acids/proteins. Alexeter RAID™ 8 immunoassays are also included for the detection of anthrax, plague, tularemia, brucellosis, smallpox, SEB, botulinum toxin, and ricin toxin, as well as screens for non-biological materials and pesticides.

Specifications

HazCat® KT7001 WMD Kit and KT7003 2.0 Pro Kit for Detection of Amino Acids/Protein Including Immunoassays for Biothreats

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD**
Amino Acids/Protein	Amino Acids/Protein	Not reported
8-Agent Biothreat Assay (Alexeter Technologies RAID™ 8)		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Tularemia	*Francisella tularensis*	1.6 million CFU/mL
Anthrax	*Bacillus anthracis*	100,000 spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	30 ng/mL
Brucellosis	*Brucella* species	1.5 million CFU/mL
Plague	*Yersinia pestis*	36,000 CFU/mL
Ricin toxin	*Ricinus communis*	6 ng/mL
Smallpox	Variola virus	1.6 million PFU***/mL
SEB	*Staphylococcus aureus*	10 ng/mL

*Reported by manufacturer.
**Approximately ~100,000 spores or 12 µg of ricin toxin
*** plaque-forming units

Assay time: *~10 minutes.*
Basis of Detection: *Presence of protein or amino acids, sample pH, immunoassay detection of pathogens and toxins.*
Required sample preparation? *Varies depending on test.*
Automatic results display? *Operator reads/interprets results.*
Unit weight: *~28 lb.*
Power: *Standard batteries.*
Cost: *KT7001 Kit – $5285. KT7003 Kit – $6100*
Additional costs: *Assays can be restocked individually; prices vary.*
Assay shelf-life: *Kit components vary; 1-5 years from date of manufacture.*

Peer-Reviewed References

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

HazTech Systems, Inc.: KT1030 HazCat® Anthrax Screening Kit

Phone: (800) 543-5487 *Manufacturer's website*: http://www.hazcat.com/

Technology Summary

The HazCat® Anthrax Screening Test Kit is designed to detect the presence of amino acids and proteins. This kit is compatible with powder samples. If the test is positive, additional tests should be performed to determine if a biological agent of concern is actually present or if the sample merely contains a harmless protein-containing substance.

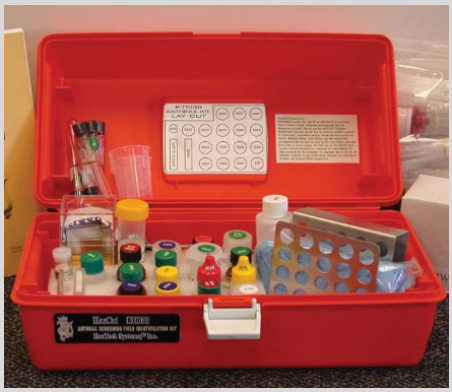
The suspected powder is resuspended in reagents supplied with the kit and introduced into the sampler. This sample preparation time is approximately 5 minutes and the use of the system does not require specialized technical skills. The response time from sample introduction to final result is 20 minutes.

An additional test provided with the kit is for the presence of organophosphates to help identify other potential toxic chemical components of suspicious white powder samples.

Peer-Reviewed References

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

Specifications



HazCat® Anthrax Screening Kit for Detection of Amino Acids/Protein

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD**
Amino Acids/Protein	Amino Acids/Protein	Not Reported

Assay time: *20 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Operator reads/interprets results.*
Unit weight: *12 lb.*
Power: *N/A.*
Cost: *Kit – $805.*
Additional costs: *$100/yr for maintenance.*
Assay shelf-life: *Indefinite with periodic maintenance test.*

Macherey-Nagel, Inc.: INDIPRO (Protein Test)

Phone: (631) 242-4249 *Manufacturer's website*: http://www.mn-net.com

Technology Summary

Like the BioCheck® Kit, this kit detects 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.

The INDIPRO kit 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. If the protein test is positive, additional tests should be performed to determine if a biological agent of concern is actually present or if the sample merely contains a harmless protein-containing substance.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system).

Peer-Reviewed References

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

Specifications

The INDIPRO Test Kit for Detection of Protein

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
INDIPRO	Protein	50 µg**
*Reported by manufacturer ** ~100 million spores		

Assay time: *~5 minutes.*
Basis of Detection: *Presence of protein; does not distinguish between biothreat and non-threat materials.*
Required sample preparation? *Minimal.*
Automatic results display? *User interprets color change.*
Unit weight: *Negligible.*
Power: *N/A.*
Cost: *Assay – $105/60 strips ($1.75 each).*
Additional costs: *None.*
Assay shelf-life: *24 months from date of manufacture.*

3M™: Clean-Trace™ Surface ATP (ATP Test)

Phone: (866) 290-0795 (Ext. 15) *Manufacturer's website*: http://solutions.3m.com

Technology Summary

The Clean-Trace™ system 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 system has two main components: a handheld luminometer to read ATP-induced luminescence and an integrated swab/reagent cartridge. Once a sample is swabbed from a surface, the swab cartridge is inserted into the luminometer and the intensity of emitted light (luminescence) is displayed as relative light units (RLU) within 30 seconds. The total time required for the entire process is <2 minutes. In general, an RLU reading of >300 is considered positive, but the threshold RLU for establishing a positive result can depend on the particular surface (e.g., a food preparation surface may have high background readings if animal or vegetable cellular materials are present). While the integrated reagent/swab is easy to use, there are no sample-treatment steps facilitate the discrimination of bacterial cells from other cells (e.g., food and skin cells). Ricin and botulinum toxins are not directly detected by this method, but detection of ATP present in any residual live cells that are associated with these toxins is possible. The Clean-Trace™ system is primarily marketed to the food industry as a rapid approach for assessing whether a surface has been cleaned properly, and this ATP detection system has not been widely tested for biothreat applications. Because spores are not metabolically active cells and do not contain high levels of ATP, they are essentially undetectable using this system unless an incubation step is performed to initiate germination and cell growth, which is not currently part of the manufacturer's procedure.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system).

Specifications

Clean-Trace™ Swab and Luminometer for Detection of ATP

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
Clean-Trace™	ATP	0.5 pg**
*Reported by manufacturer **Approximately 10,000 organisms/mL; note that spores may not be detectable using this product		

Assay time: *<2 minutes.*
Basis of Detection: *Presence of ATP (requires live, metabolically active biological material); does not distinguish between biothreat and non-threat materials.*
Required sample preparation? *No.*
Automatic results display? *User interprets numerical readout.*
Unit weight: *2 lb.*
Power: *Battery or AC.*
Cost: *Assay – $250/100 pack ($2.50 each); instrument – $2900; docking station & carrying case – $265.*
Additional costs: *None.*
Assay shelf-life: *10 months (2-8°C); 28 days (21°C); 7 days (25°C) from date of manufacture.*

Peer-Reviewed References

Omidbakshs, N., Ahmadpour, F. and Kenny, N. How Reliable Are ATP Bioluminescence Meters in Assessing Decontamination of Environmental Surfaces in Healthcare Settings? *PLoS ONE*. **2014**. 9, e99951. DOI: 10.1371/journal.pone.0099951.

This study compared four different ATP meters against pure solutions of increasing concentrations of ATP as well as serial dilutions of *Staphylococcus aureus* broth cultures. All tested detection platforms were based on ATP bioluminescence measurements with the user readout being reported in RLU. The objective of this study was to, for each of the ATP instruments, determine the sensitivity, limits of linearity, and correlation of measurements to known concentrations of bacteria, and discern whether disinfecting agents interfered with instrument performance. The authors tested the Kikkoman Lumitester PD-20 from Luminultra Technologies Ltd. (with LuciPac Pen swabs), EnSURE Hygiene Meter – ATP-205 from Hygiena/Scigiene Corporation (with ATP3000 SuperSnap swabs), Clean-Trace NG Luminometer UNG2 from 3M Company (with Surface ATP – UXL100 swabs), and Charm novaLUM from Charm Sciences Inc. (with PocketSwab Plus ATP swabs). The 3M instrument had a linear range of detection from 10^{-11} up to 10^{-4} M of ATP solution (ATP diluted in water). Loss of linearity was observed at 10^{-3} M and higher concentrations. The 3M instrument had a linear range of detection from 10^{3} up to 10^{10} CFUs of *S. aureus,* and a lower LOD of 8.98×10^{2} CFU. However, significant signal quenching was observed when testing a 10^{-8} M solution of ATP in the presence of the following disinfectants: cavicide, PCS 1000, SaniCloth, Accel TB, CleanCide, Clorox H_2O_2 Wipes, Clorox Clean-up Disinfectant, and bleach. Limited signal reduction was observed when 70% isopropanol, 0.5% H_2O_2, or Virox 5 RTU were used as the disinfectant.

Industrial Hygiene Consulting, Corporation: Bio-Reveal™ (ATP Test)

Phone: (866) 989-5567 *Manufacturer's website*: http://www.bio-reveal.com/home.html

Technology Summary

The Bio-Reveal™ ATP detection system is a handheld device that semi-quantitatively determines the amount of biomass in a sample by measuring levels of ATP. 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. There are two main components to the Bio-Reveal system, a handheld luminometer and sampling swabs. In addition, there are two types of self-contained swabs—swabs for liquid samples and swabs for surfaces. After obtaining the sample on the swab, the user returns the swab to the swab containment device and breaks a snap valve to release the assay reagent. The swab is then introduced into the handheld luminometer and the instrument provides readout values in RLU.

The Bio-Reveal™ ATP detection system is primarily marketed to remediation services and clinical settings for the purpose of building environmental management (e.g., mold remediation or bio-contamination assessment). This instrument has not been tested specifically for biothreat applications. Careful consideration must be made in using this product for suspected biothreat spore testing because spores are not metabolically active cells and do not contain high levels of ATP; thus, they are essentially undetectable using this system unless an incubation step is performed to initiate germination and cell growth, which is not currently part of the manufacturer's procedure.

Specifications

Bio-Reveal™ Swab and Luminometer for Detection of ATP

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
Bio-Reveal™	ATP	0.5 pg**

*Reported by manufacturer
**Approximately 10,000 organisms/mL; note that spores may not be detectable using this product

Assay time: *Less than 1 minute.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes.*
Unit weight: *0.5 lb.*
Power: *2 x AA batteries.*
Cost: *Assay – $250/100 surface swabs ($2.50 each); starter kit with instrument –$1295.*
Additional costs: *None.*
Assay shelf-life: *4 weeks (room temperature); 12 months (4° C) from date of manufacture.*

Peer-Reviewed References

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

New Horizons Diagnostics, Inc.: PROFILE® 1 (ATP Test)

Phone: (443) 543-5755 *Manufacturer's website*: http://www.nhdiag.com

Technology Summary

The PROFILE® 1 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 microbial cells from bacterial spores and human cells through the use of sample preparation methods that selectively remove them using supplied reagents. The system has two main components: a microluminometer to read ATP-induced luminescence and a filtration device and cuvette combined (i.e., filtravette) 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 optical reader and the intensity of emitted light (luminescence) is recorded. Because they are proteins and not living cells, ricin and botulinum toxins are not detected by this method unless residual live cells associated with these toxins are present.

Specifications

PROFILE® 1 Kit for Detection of ATP

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
PROFILE® 1	ATP	~10,000 spores
* Reported in peer-reviewed reference ** Approximately 2000-10,000 spores/mL		

Assay time: *~15 minutes (longer growth times can be used to improve sensitivity).*
Basis of Detection: *Presence of ATP (requires live, metabolically active biological material); does not distinguish between biothreat and non-threat materials.*
Required sample preparation? *Moderate.*
Automatic results display? *User interprets numerical readout.*
Unit Weight: *<1 lb.*
Power: *9V battery (1000+ reads) or AC.*
Cost: *Assay – $450 (reagents and materials for 100 tests; $4.50 each); instrument – $5000 (includes carrying kit, microluminometer, filtravette, cell concentrator, and pipettor).*
Additional costs: *None.*
Assay shelf-life: *12 months from date of manufacture (some reagents require refrigeration).*

Spores do not contain high levels of ATP and are essentially undetectable using the PROFILE® 1 kit unless an incubation step (reagents are supplied with the kit) is performed to initiate cell growth. When analyzing a suspected anthrax spore powder the sample must be incubated in growth medium for approximately 15 minutes.

Peer-Reviewed References

Poore, C.; Clark, P.; Emanuel, P. A. An Evaluation of Suspicious Powder Screening Tools for First Responders. *J. Hazard. Mater.* **2009**, *172*, 559-65. DOI: 10.1016/j.jhazmat.2009.05.142.

This study compared the effectiveness of three general biological indicator tests to differentiate suspicious (but harmless) powders from biological threat agents. Test samples included *Bacillus anthracis* (Sterne strain) (washed 2 or 4 times to reduce extraneous DNA and cellular protein on spore surfaces), *Yersinia pestis* (A1122 strain), and purified ricin. These studies were performed with biothreat samples added directly to the kit solutions; thus, LODs are not reported as substance per milliliter (i.e., concentration). The 20/20 Gene Systems BioCheck® Powder Screening kit (protein test) could detect 1×10^8 CFU of *Bacillus anthracis* (4X wash), 1×10^7 CFU of *Bacillus anthracis* (2X wash), 1×10^7 CFU of *Yersinia pestis*, and 100 µg of ricin. The GenPrime Prime Alert® (DNA test) could detect 2×10^{10} CFU of *Bacillus anthracis* (4X wash), 1×10^9 CFU of *Bacillus anthracis* (2X wash), and 1×10^8 CFU of *Yersinia pestis.* The New Horizons Diagnostics PROFILE® 1 (ATP test) could detect 1×10^4 CFU of *Bacillus anthracis* (both 2X and 4X wash) and 1×10^6 CFU of *Yersinia pestis.* Because purified ricin was used, the Prime Alert® and PROFILE® 1 were unable to detect ricin as neither DNA nor ATP were present. Powders were also tested after spiking with biological agents. In general, all kits gave positive results for powders that contained the targeted substance (e.g., protein or cells), but some powders caused interferences and impacted the sensitivity as discussed in the Conclusions section of the paper.

Min, J.; Lee, J.; Deininger, R. A. Simple and Rapid Method for Detection of Bacterial Spores in Powder Useful for First Responders. *Journal of Environmental Health* **2006,** *68* (8).

This study evaluated how changing variables in the protocol used by the New Horizons Diagnostics PROFILE® 1 impact the speed and sensitivity of detection of ATP. Sample sizes of 1 mg *Bacillus thuringiensis* spores were used to investigate the impact of germination time (2, 5, 15 minutes), germination temperature (37°C vs. 55°C), nutrient type (brain heart infusion [BHI] medium vs. tryptic soy broth), and nutrient concentration (0.5X, 1X, or 2X strength). The germination step converts the dormant spores to live vegetative cells that contain ATP. Germination also improves the method sensitivity during the short nutrient growth step, which increases the number of cells. The protocol involves adding an aliquot of the sample/buffer solution to a disposable filter tube, adding somatic cell-releasing agent and filtering to remove non-bacterial cells, which leaves the *Bacillus* cells on the filter surface. Bacterial cell-releasing agent is added to lyse the *Bacillus* cells, then luciferin-luciferase reagent is added, which binds with ATP and generates light that is read by a luminometer. Optimal conditions were determined as 37°C germination temperature, 15 minute germination time, and 1X strength of BHI.

Lee, J-Y.; Deininger, R. A. A Rapid Screening Method for the Detection of Viable Spores in Powder Using Bioluminescence**.** *Luminescence* **2004**, *19,* 209–211. DOI: 10.1002/bio.775.

This paper explores different sample preparation methods to speed the germination and subsequent detection of ATP in *Bacillus thuringiensis* (a surrogate for *Bacillus anthracis*) spores. The ATP bioluminescence method was used to detect the presence of spores in powder. Only spore-containing powder samples provided a dramatic increase in the bioluminescence signal after heat shock, which induces germination of spores and results in vegetative cells that have ATP. At 37°C the assay required approximately 15 minutes, while at 50°C spore germination was faster, resulting in a protocol that only required about 2 minutes. For the PROFILE® 1 system, the authors reported the detection of <100 spores in a 50 µL sample (i.e., 2000 spores/mL).

GenPrime, Inc.: Prime Alert® Microbe Screen (DNA Test)

Phone: (866) 624-9855 *Manufacturer's website*: http://www.genprime.com

Technology Summary

The Prime Alert® System is designed to test for biological material in a white powder sample based on the presence of DNA or double-stranded RNA. This system is also distributed through Smiths Detection.

The Prime Alert® System detects any type of DNA-based microbial or cellular material, not just anthrax or other biothreat DNA. The test is not specific to an individual organism and only indicates if the sample contains DNA or some types of RNA. The assay consists of a binding dye that fluoresces only when bound to DNA or certain RNA. Fluroescence 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 optical reader before each use. Following a 1-minute reader calibration step that involves reading an included calibration solution, the assay takes approximately 5 minutes to complete. To perform the test, cell prep solution is added to a glass sample vial dropwise from a dropper bottle, and dye solution is added using a provided transfer pipette. Then the powder sample is added to the cell prep solution dropper bottle and mixed by shaking. Four drops of the resulting solution are added to the sample vial and then the vial is placed in the reader for analysis. The operator interprets the numerical readout based on a cutoff specified by the vendor.

The management system governing the manufacture of this product is ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices).

Specifications

Prime Alert® Optical Fluorescence Reader and Contents of the Microbe Screen Kit for Detection of DNA

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
Prime Alert® Microbe Screen	DNA	~1 million spores
*Reported by manufacturer		

Assay time: *~5 minutes.*
Basis of Detection: *Presence of DNA; does not distinguish between biothreat and non-threat materials.*
Required sample preparation? *Minimal.*
Automatic results display? *User interprets numerical readout.*
Unit weight: *1 lb (optical reader); 12 lb (complete kit).*
Power: *4 AAA batteries (2000+ readings).*
Cost: *Assay – $70; Starter Kit – $12,000 (includes fluorometer and supplies for five microbe [DNA] screens, five immunoassay toxin screens, and two training kits).*
Additional costs: *None.*
Assay shelf-life: *12 months from date of manufacture.*

Peer-Reviewed References

Poore, C.; Clark, P.; Emanuel, P. A. An Evaluation of Suspicious Powder Screening Tools for First Responders. *J. Hazard. Mater.* **2009**, *172*, 559-65. DOI: 10.1016/j.jhazmat.2009.05.142.

This study compared the effectiveness of three general biological indicator tests to differentiate suspicious (but harmless) powders from biological threat agents. Test samples included *Bacillus anthracis* (Sterne strain) (washed 2 or 4 times to reduce extraneous DNA and cellular protein on spore surfaces), *Yersinia pestis* (A1122 strain), and purified ricin. These studies were performed with biothreat samples added directly to the kit solutions; thus, LODs are not reported as substance per milliliter (i.e.,

concentration). The 20/20 Gene Systems BioCheck® Powder Screening kit (protein test) could detect 1×10^8 CFU of *Bacillus anthracis* (4X wash), 1×10^7 CFU of *Bacillus anthracis* (2X wash), 1×10^7 CFU of *Yersinia pestis*, and 100 µg of ricin. The GenPrime Prime Alert® (DNA test) could detect 2×10^{10} CFU of *Bacillus anthracis* (4X wash), 1×10^9 CFU of *Bacillus anthracis* (2X wash), and 1×10^8 CFU of *Yersinia pestis.* The New Horizons Diagnostics PROFILE® 1 (ATP test) could detect 1×10^4 CFU of *Bacillus anthracis* (both 2X and 4X wash) and 1×10^6 CFU of *Yersinia pestis.* Because purified ricin was used, the Prime Alert® and PROFILE® 1 were unable to detect ricin as neither DNA nor ATP were present. Powders were also tested after spiking with biological agents. In general, all kits gave positive results for powders that contained the targeted substance (e.g., protein or cells), but some powders caused interferences and impacted the sensitivity as discussed in the Conclusions section of the paper.

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

Smiths Detection: HazMatID™ 360 (FTIR)

Phone: (800) 297-0955 *Manufacturer's website*: http://www.smithsdetection.com

Technology Summary

The HazMatID™ 360 is a hand-portable FTIR spectrometer for the identification of unknown solids, liquids, pastes, or gels. 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 HazMatID™ 360 can identify over 32,000 substances, including many white powders, and can be upgraded with the purchase of additional libraries. 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.

The presence of biological material is tentatively indicated, primarily based on the sample protein content and the software alerts the user when a sample has a protein content of approximately 10% or more, indicating a sample of potential biological origin.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system).

Specifications

HazMatID™ 360 Hand-Portable FTIR With Integral Sample Press

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
Not Applicable	Protein	Present at >10%
*Approximate value based on published literature		

Assay time: *<2 minutes.*
Basis of Detection: *Almost all substances absorb infrared light at different wavelengths or frequencies and can be identified by their resultant spectrum. The presence of protein can be used as an indicator that a biothreat may be present.*
Required sample preparation? *No.*
Automatic results display? *User compares spectral library matches with sample spectrum.*
Unit weight: *23 lb.*
Power: *Internal rechargeable battery, AC, or automobile cigarette lighter.*
Cost: *Instrument – $55,000.*
Additional costs: *ExtractIR; Bluetooth; additional spectral libraries; software upgrade; optional wireless capability to interface with Responder RCI™ Raman spectrometer.*
Assay shelf-life: *N/A.*

Peer-Reviewed References

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

Smiths Detection: HazMatID™ Elite (FTIR)

Phone: (800) 297-0955 *Manufacturer's website*: http://www.smithsdetection.com

Technology Summary

The HazMatID™ Elite FTIR is specifically designed for ease-of-use in potentially contaminated zones 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, optional, touch-to-sample diamond attenuated total reflection (ATR) sensor is available for analysis of pooled liquid and surface films and to enable robotics applications. The 4.3-in. color display has high visibility in direct sunlight. Easy-to-use software and on-screen instructional graphics guide the user through 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. The Elite is also minimally affected by vibrations and movement during sample acquisition. An embedded radio frequency (RF) modem enables line-of-sight communication of up to 1 km for data transfer and remote operation with an optional repeater to extend the range. A global positioning system (GPS) is also included. The device base 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.

The presence of biological material is tentatively indicated, primarily based on the sample protein content and the software alerts the user when a sample has a protein content of approximately 10% or more, indicating a sample of potential biological origin. The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system).

Specifications

HazMatID™ Elite Handheld FTIR With Integral Sample Press

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
Not Applicable	Protein	Present at >10%
*Approximate value based on published literature		

Assay time: *<2 minutes.*
Basis of Detection: *Almost all substances absorb infrared light at different wavelengths or frequencies and can be identified by their resultant spectrum. The presence of protein can be used as an indicator that a biothreat may be present.*
Required sample preparation? *No.*
Automatic results display? *User compares spectral library matches with sample spectrum.*
Unit weight: *5 lb.*
Power: *Internal rechargeable battery or disposable123A batteries (4 hours of operation). AC and vehicle power options.*
Cost: *Instrument – $45,000.*
Additional costs: *Optional software package for data reprocessing on a remote PC using 35,000 spectra library; optional RF repeater for increased wireless range.*
Assay shelf-life: *N/A.*

Peer-Reviewed References

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

Smiths Detection: HazMatID™ Ranger (FTIR)

Phone: (800) 297-0955 *Manufacturer's website*: http://www.smithsdetection.com

Technology Summary

The HazMatID™ Ranger is a FTIR system designed for handheld, backpack, or robot portability and ease-of-use. Samples are measured by firmly pressing the diamond 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 in a decontamination solution 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 about 3 hours and the PDA battery operates for about 8 hours. The standard library contains spectra of approximately 4400 materials, and the optional library contains spectra of approximately 32,000 materials. An additional option is wireless capability that allows communication with a PC 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.

The presence of biological material is tentatively indicated, primarily based on the sample protein content and the software alerts the user when a sample has a protein content of approximately 10% or more, indicating a sample of potential biological origin. The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system).

Specifications

HazMatID™ Ranger Handheld FTIR

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
Not Applicable	Protein	Present at >10%
*Approximate value based on published literature		

Assay time: *<2 minutes.*
Basis of Detection: *Almost all substances absorb infrared light at different wavelengths or frequencies and can be identified by their resultant spectrum. The presence of protein can be used as an indicator that a biothreat may be present.*
Required sample preparation? *No.*
Automatic results display? *User compares indicated spectral library matches with sample spectrum.*
Unit weight: *6.5 lb.*
Power: *Internal battery (>3 hours of operation).*
Cost: *Instrument – $35,000.*
Additional costs: *Optional wireless capability to interface with Responder RCI™ Raman spectrometer; optional >32,000 material library (vs. standard >4400).*
Assay shelf-life: *N/A.*

Peer-Reviewed References

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

Thermo Scientific: TruDefender® FT and FTi (FTIR)

Phone: (480) 532-6171 *Manufacturer's website*: http://www.ahurascientific.com

Technology Summary

The TruDefender® FT and FTi are handheld FTIR 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 FTIR in general 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, the 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 by a user wearing a Level A protective suit. For decontamination, the unit can be immersed in water and household bleach (5% sodium hypochlorite).

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system).

Specifications

TruDefender® Handheld FTIR

General Biological Indicator Test		
Assay	**Biological Indicator**	**LOD***
Not Applicable	Protein	Present at >10%
*Approximate value based on published literature		

Assay time: *<2 minutes.*

Basis of Detection: *Almost all substances absorb infrared light at different wavelengths or frequencies and can be identified by their resultant spectrum. The presence of protein can be used as an indicator that a biothreat may be present.*

Required sample preparation? *No.*

Automatic results display? *User compares indicated spectral library matches with sample spectrum.*

Unit weight: *2.9 lb.*

Power: *Removable, rechargeable battery, or 123Aa SureFire™ batteries (>4 hours operation).*

Cost: *Instrument – $45,000* (FT); *$46,500 (FTi).*

Additional costs: *Software/library updates are included with active support contracts.*

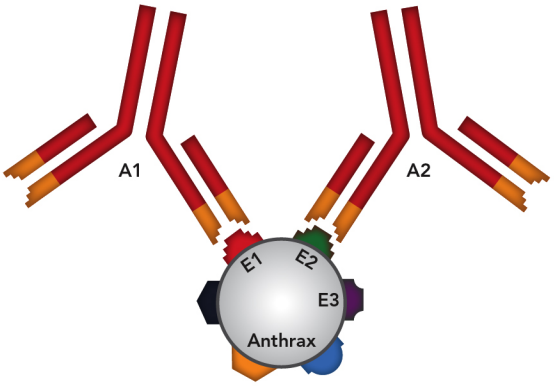
Assay shelf-life: *N/A.*

Peer-Reviewed References

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

4.0 Immunoassays and Miscellaneous Technologies

Biodetection immunoassays 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 such as anthrax or ricin.



Schematic drawing of two different antibodies (A1 and A2) binding to two different regions (E1 and E2) on a biothreat 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 manually (with your eye) or using an electronic optical reader. LFAs require liquid samples; thus, they typically require a swab collection kit that solubilizes or suspends material from the swab into a buffer. After a sample is collected a few drops of sample solution are added to the sample inlet window. The sample solution is then wicked across the strip and through a reagent zone that contains dye-labeled antibodies specific to the threat agent. Threat agents 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 colored 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 typically be obtained in only 5 to 15 minutes. However, most immunoassays are thousands of times less sensitive than PCR. Typical LODs for immunoassays range from 1 million to 10 million spores or microbes per milliliter. Automated sample processing and detection systems can improve sensitivity by 10-fold or more.

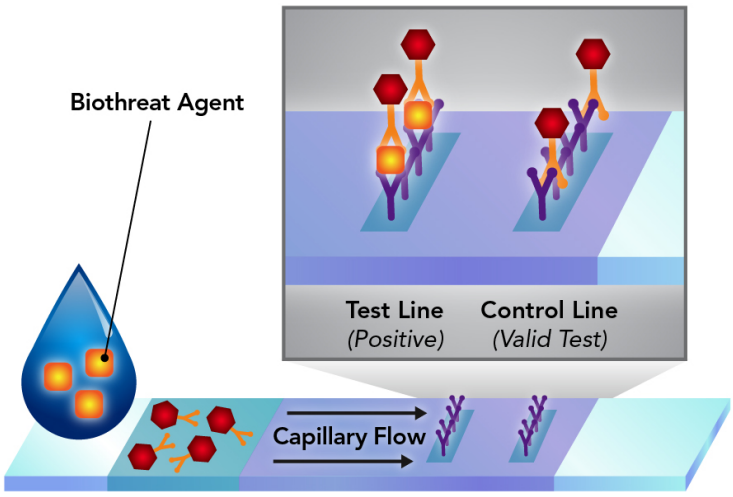


Illustration of a typical lateral flow immunoassay.

False positives can occur if closely related biothreats are present (like non-pathogenic relatives of *Bacillus anthracis* that are found naturally in the environment). Some commonly encountered substances (e.g., suspicious powders) can cause some assays to give

false-positive results, particularly if too much sample is used. Immunoassays can also be subject to false negatives resulting from interferents and highly concentrated samples. For example, if there are more biothreat agents than dye-labeled antibody reagents, unlabeled biothreat agents may bind to the test line and effectively block the labeled biothreat agents from binding. In this situation, the sample line would be negative and the control line may or may not be positive (depending on the assay design).

Single Agent Tests

- Assay cost: ~$2 to $65
- Optical reader cost: $3225 to $11,500 (optional for most)
- Examples:
  - BADD™ (AdVnt Biotechnologies)
  - BioDetect™ (Alexeter Technologies) with portable and handheld optional optical readers
  - ENVI Assay System Gold (Environics) with optional optical reader
  - KDTB GOLD (NBC-SYS)
  - Smart™-II (New Horizons Diagnostics)
  - RAMP® (Response Biomedical) with required optical fluorescence reader
  - BioThreat Alert® (Tetracore) with optional optical reader

Multiple Agent Tests (2-8 agent assays)

- Assay cost: ~$75 to $125
- Optical reader cost: $6900 (only available for NIDS®)
- Examples:
  - Pro Strips™ (AdVnt Biotechnologies)
  - RAID™ (Alexeter Technologies)
  - NIDS® (ANP Technologies) with optional optical reader
  - IMASS (BBI Detection)
  - Toxin Screen (GenPrime)

Automated Immunoassay Systems

- Assay cost: $16 (1-agent) to $250 (8-agent) to $564 (5-agent toxin)
- Examples:
  - Portable Toxin Detector 5-agent toxin assay (Bruker Daltronics)
  - PR2 1800 electrochemiluminescence immunoassay system (Meso Scale Defense)
  - Zephyr® 1-agent assay (Path Sensors)
  - BioHawk® 8-agent assay (Research International)
  - RAPTOR™ 4-agent assay (Research International)

Miscellaneous Systems

- Assay cost: $200-$400
- Examples:

- MENTOR 100 and Lab-in-the-Box immunoassay- and nucleic acid-based probe Nuclear Magnetic Resonance (NMR) biodetectors (Menon Biosensors)
- PR2 1800 multiplexed electrochemiluminescent immunoassay detection system in 96-well plate format (Meso Scale Defense)
- TIRF Sense and iTIRF immunoassay- and nucleic acid-based probe total internal reflectance fluorescence biodetectors (TIRF Labs).

AdVnt Biotechnologies, LLC: BADD™

Phone: (888) 223-3269 *Manufacturer's website*: http://www.advnt.org/

Technology Summary

The AdVnt Biowarfare Agent Detection Devices (BADD™) are LFAs for the detection of specific biothreats. These devices test for one agent 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 can be seen in 15 minutes. However, with concentrated samples a positive result may be obvious after only 3 minutes.

One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system) and ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices). This product has received a "Certified" classification (consistently proven effectiveness, with high confidence of enduring effectiveness) by DHS as part of its SAFETY Act of 2002 (www.safetyact.gov).

Specifications

BADD™ 1-agent immunoassay cartridges

Biothreat 1-Agent Assays		
Disease/ Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	15,000-83,000 spores/mL (strain dependent)
Botulism (botulinum toxin)	*Clostridium botulinum*	33 ng/mL (BoNT A), 500 ng/mL (BoNT B)**
Plague	*Yersinia pestis*	100,000 CFU/mL
Ricin toxin	*Ricinus communis*	10 ng/mL
SEB	*Staphylococcus aureus*	10 ng/mL
Tularemia	*Francisella tularensis*	1.5 million CFU/mL

*Reported by manufacturer.
**Botulinum neurotoxin A and botulinum neurotoxin B

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *User interprets presence/absence of a line.*
Unit weight: *Negligible.*
Power: *N/A.*
Cost: *Assay – $257/10 pack ($26 each).*
Additional costs: *None.*
Assay shelf-life: *24 months from date of manufacture.*

Peer-Reviewed References

Slotved H. C., Sparding N., Tanassi J. T., Steenhard N. R., Heegaard N. H. Evaluating 6 ricin field detection assays. *Biosecurity and bioterrorism: biodefense strategy, practice, and science*. **2014**, Jul-Aug;12(4):186-9. DOI: 10.1089/bsp.2014.0015

This study compared eight commercially available ricin detection assays using pure ricin toxin. The eight assays compared are LFDs from AdVnt Biotechnologies (BADD™, Pro Strips™), Environics (ENVI Assay System), Alexeter Technologies (RAID™ 8, RAID™ DX, RAID™ TOX), Tetracore (BioThreat Alert®), BBI Detection (IMASS™). Ricin concentrations ranging from 0.625 to 20 ng/mL were tested with respect to manufacturers' stated detection limits. BADD™ and Pro Strips™ did not

detect ricin at 20 ng/mL. The ENVI Assay System detected ricin at 20 ng/mL, gave mixed results at 10 ng/mL, and did not detect ricin at 5 ng/mL. The RAID™ 8, RAID™ DX and RAID™ TOX did not detect ricin at 20 ng/mL. The BioThreat Alert® detected ricin at 20, 10, and 5 ng/mL, but not at 2.5 or 1.5 ng/mL. The IMASS™ device detected ricin at 20 and 10 ng/mL but not at lower concentrations tested. The only assay evaluated, which was able to detect ricin at the detection limit stated by the manufacturer, was the BioThreat Alert®.

Peckham, G. D.; Hew, B. E.; Waller, D. F.; Holdaway, C.; Jen, M. Amperometric Detection of *Bacillus anthracis* Spores: A Portable, Low-Cost Approach to the ELISA. *Int. J. Electrochem.* **2013**, *2013,* Article 803485. DOI: 10.1155/2013/803485. http://www.hindawi.com/journals/ijelc/2013/803485/

This investigation compared an antibody-based method using amperometric signal generation to enzyme-linked immunosorbent assays (ELISAs) and LFAs for detecting *Bacillus anthracis* (Sterne strain) spores. Tetracore's ELISA assay kits were used. The LFAs included BADD™, Smart™-II, and BioThreat Alert®. At least 15 of each LFA were tested with spore concentrations of 10^4 CFU/mL to 5 x 10^6 CFU/mL. All LFAs detected 5 x 10^6 CFU/mL in greater than >90% of trials. Performance decreased with decreasing spore concentration (<80% for 10^6 CFU/mL and <60% for 5 x 10^5 CFU/mL). Smart™-II was the only immunoassay tested that detected samples with 10^5 CFU/mL, but this was achieved in <40% of trials. BioThreat Alert® was reported to be the easiest to interpret with a dark, highly contrasted test line. The BADD™ readout was reported to be very faint, even at high spore concentrations. ELISA had 100% positive detection for all spore concentrations, however each assay took over 6 hours to complete. Amperometry, which required just over 1 hour to complete, detected spores in >90% of trials at >10^5 CFU/mL; however, performance decreased with decreasing spore concentration (86% for 5 x 10^4 CFU/mL and 47% for 10^4 CFU/mL).

Slotved, H.-C.; Tanassi, J. T.; Sparding, N.; Lindqvist, A.; Steenhard, N. R.; Heegaard, N. H. H. Botulinum Toxin Field Assays Evaluated Using Cosmetic Botox Preparations**.** *Biosecurity and bioterrorism: biodefense strategy, practice, and science.* **2013**, *11,* 280-286. DOI: 10.1089/bsp.2013.0050.

This study compared several different botulinum detection technologies using botulinum toxins (BoNTs) A and B, as well as four pharmaceutical-grade cosmetic botulinum toxin preparations. An ELISA method was used as a baseline and compared to an immunoaffinity column and LFAs from AdVnt Biotechnologies (1-agent BADD™ and 5-agent ProStrips), Environics (ENVI Assay system), and Alexeter (RAID DX kit, which contains an 8-agent RAID 8 LFA and a 3-agent RAID TOX LFA). Relatively low concentrations of BoNTs (both commercial and pharmaceutical sources) were used and were not detected by most of the LFAs. BADD™ did not detect pharmaceutical BoNT A (100 ng/mL), commercial BoNT A (50 ng/mL), or commercial BoNT B (500 ng/mL and 10,000 ng/mL). ProStrips did not detect commercial BoNT A (50 ng/mL and 10,000 ng/mL), commercial BoNT B (10,000 ng/mL), or pharmaceutical BoNT A (100 ng/mL). The ENVI Assay System gave one positive result and one negative result with commercial BoNT A (10,000 ng/mL), but did not detect pharmaceutical BoNT A (100 ng/mL and 27.5 ng/mL). The RAID 8 and RAID TOX did not detect pharmaceutical BoNT A (27.5 ng/mL and 13.75 ng/mL), although those concentrations are below the manufacturer stated LOD for BoNT.

Gessler, F.; Pagel-Wieder, S.; Avondet, M-A. Bohnel, H. Evaluation of Lateral Flow Assays for the Detection of Botulinum Neurotoxin Type A and Their Application in Laboratory Diagnosis of Botulism. *Diagn. Microbiol. Infect. Dis*. **2007**, *57*, 243–249. DOI: 10.1016/j.diagmicrobio.2006.07.017.

This study evaluated BioThreat Alert®, Smart™-II, BADD™, and RAMP® assays for their ability to detect botulinum neurotoxin in three forms: purified botulinum neurotoxin A (BoNT A), toxin complex, and toxin in the supernatant of a *Clostridium botulinum* culture. Only BADD™ and RAMP® detected purified BoNT A; LODs were 100 ng/mL for BADD™ and 50 ng/mL for RAMP®. All assays detected the BoNT A complex and SMART™-II and BioThreat Alert had greater sensitivity for the BoNT A complex (10 ng/mL) than BADD™ (100 ng/mL) and RAMP® (250 ng/mL). For the *Clostridium botulinum* culture samples, the concentration of active toxin was estimated using a mouse lethality assay. The lowest concentration of toxin in culture medium that the BioThreat Alert®, Smart™-II, and BADD™ could detect was 100 Minimal mouse Lethal Doses (MLD)/mL, while RAMP® could detect only 2500 MDL/mL. BADD™ gave a false-positive result for a culture medium that did not contain *Clostridium botulinum* or botulinum toxin.

King, D.; Luna, V.; Cannons, A.; Cattani, J.; Amuso, P. Performance Assessment of Three Commercial Assays for Direct Detection of *Bacillus anthracis* Spores. *J. Clin. Microbiol*. **2003**, *41*, 3454–3455. DOI:10.1128/JCM.41.7.3454-3455.2003.

This brief study by the Florida Department of Health Laboratory evaluated three immunoassay tests including BioThreat Alert®, Osborne Scientific's 1st generation BADD (Note: Osborne's biothreat product line was acquired by AdVnt in 2003 and the current assay is 3rd generation), and Smart™-II. The tests were evaluated for *Bacillus anthracis* (Pasteur strain) detection at quantities ranging from 10^2 to 10^6 spores (the concentration of the test samples and volume of sample applied were not reported). Only 2 to 8 samples were tested at each concentration. All test kits could detect *Bacillus anthracis* at 10^6 spores. BADD™ and Smart™-II could detect 10^5 spores; however, BioThreat Alert® detected 10^5 spores only once in eight separate assays. None of the assays could detect fewer than 10,000 spores. Tests were allowed to develop for 15 minutes, although positive results were apparent within 5 minutes. *Bacillus cereus* and *Bacillus thuringiensis* (non-threat near neighbors that could potentially result in a false-positive) were also tested twice for each test strip. No false positives were observed for the BADD™ or BioThreat Alert® tests; however, the Smart™-II tests yielded one false-positive result for *Bacillus thuringiensis*.

Zasada A.A.; Formińska, K.; Zacharczuk, K.; Jacob, D., and Grunow, R. "Comparison of Eleven Commercially Available Rapid Tests for Detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*." *Letters in Applied Microbiology*. **2015**, DOI: 10.1111/lam.12392.

This study compared 11 different commercially available rapid tests for detecting *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*. For each detection platform, the manufacturer's instructions were followed and tested against each of the aforementioned bacteria in concentrations ranging from 10^3 to 10^8 CFU/mL. *Y. pestis* was detected at $\geq 10^6$ CFU/mL for the New Horizons Diagnostics SMART-II LFA, at $\geq 10^7$ CFU/mL for the Tetracore BioThreat Alert LFA, and at $\geq 10^8$ CFU/mL for the AdVnt BADD LFA. *Bacillus anthracis* was detected at $\geq 10^8$ spores/mL for the New Horizons Diagnostics SMART-II LFA and the Tetracore BioThreat Alert LFA, but the AdVnt BADD LFA did not detect this concentration. *F. tularensis* was detected at $\geq 10^7$ CFU/mL for the New Horizons Diagnostics SMART-II LFA and at $\geq 10^8$ CFU/mL for the Tetracore BioThreat Alert LFA.

AdVnt Biotechnologies, LLC: Pro Strips™

Phone: (888) 223-3269 *Manufacturer's website*: http://www.advnt.org/

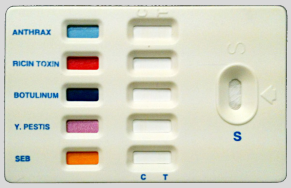
Technology Summary

AdVnt Pro Strips™ are, basically, five BADD™ assays bundled into a single cartridge. In the Pro Strips™ assay, one sample is automatically divided among five different assays. Following sample collection with the included all-in-one swab kit, approximately 10 to 11 drops (~0.4 mL) of the solution are added to the sample inlet window. Results are read in a separate window for each agent tested.

One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system) and ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices). This product has received a "Certified" classification (consistently proven effectiveness, with high confidence of enduring effectiveness) by DHS as part of its SAFETY Act of 2002 (www.safetyact.gov).

Specifications



Pro Strips™ 5-Agent Immunoassay Cartridge

Biothreat 5-Agent Assay		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	15,000 – 83,000 spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	33 ng/mL (BoNT A) 500 ng/mL (BoNT B)
Plague	*Yersinia pestis*	100,000 CFU/mL
Ricin toxin	*Ricinus communis*	10 ng/mL
SEB	*Staphylococcus aureus*	10 ng/mL
*Reported by manufacturer.		

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *User interprets presence/absence of a line.*
Unit weight: *Negligible.*
Power: *N/A.*
Cost: *Assay – $735/10 pack ($73 each; $15/agent).*
Additional costs: *None.*
Assay shelf-life: *24 months from date of manufacture.*

Peer-Reviewed References

Slotved H. C., Sparding N., Tanassi J. T., Steenhard N. R., Heegaard N. H. Evaluating 6 ricin field detection assays. *Biosecurity and bioterrorism: biodefense strategy, practice, and science*. **2014**, Jul-Aug;12(4):186-9. DOI: 10.1089/bsp.2014.0015

This study compared eight commercially available ricin detection assays using pure ricin toxin. The eight assays compared are LFDs from AdVnt Biotechnologies (BADD™, Pro Strips™), Environics (ENVI Assay System), Alexeter Technologies (RAID™ 8, RAID™ DX, RAID™ TOX), Tetracore (BioThreat Alert®), BBI Detection (IMASS™). Ricin concentrations ranging from 0.625 to 20 ng/mL were tested with respect to manufacturers' stated detection limits. BADD™ and Pro Strips™ did not detect ricin at 20 ng/mL. The ENVI Assay System detected ricin at 20 ng/mL, gave mixed results at 10 ng/mL, and did not detect ricin at 5 ng/mL. The RAID™ 8, RAID™ DX and RAID™ TOX did not detect ricin at 20 ng/mL. The BioThreat Alert® detected ricin at 20, 10, and 5 ng/mL, but not at 2.5 or 1.5 ng/mL. The IMASS™ device detected ricin at 20 and 10 ng/mL but not at lower concentrations tested. The only assay evaluated, which was able to detect ricin at the detection limit stated by the manufacturer, was the BioThreat Alert®.

Alexeter Technologies, LLC: BioDetect™

Phone: (877) 591-5571 *Manufacturer's website*: http://www.alexeter.com

Technology Summary

Alexeter BioDetect™ Test Strips are standard 1-agent LFAs. Each box of 25 assays includes 15 mL of buffer, 5 swabs, and 5 plastic vials. 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 manually or with the Alexeter Guardian Reader™, Defender Test Strip Reader (TSR™).

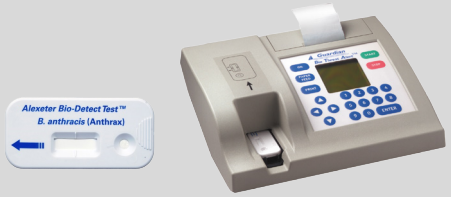
One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded. For the toxin assays, the manufacturer recommends running an additional test with a 1:40 dilution of the sample to minimize the possibility of using too much sample (i.e., the "hook effect," which can yield a false-negative result at high threat agent concentrations).

For increased accuracy and sensitivity, these test strips can be used with an optical reader (available separately). The primary advantages of using an optical 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.

Peer-Reviewed References

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

Specifications



BioDetect™ Test Strip *Guardian™ Reader*

The Defender TSR™

BioDetect 1-Agent Immunoassay Cartridgeand Optical Readers

Biothreat 1-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	1-5 million spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	30 ng/mL (Toxin A) 500 ng/mL (Toxin B)
Brucellosis	*Brucella* species	200,000-1.3 million CFU/mL
Plague	*Yersinia pestis*	500,000-1 million CFU/mL
Ricin toxin	*Ricinus communis*	5-10 ng/mL
Smallpox	Variola virus	20-50 µg/mL
SEB	*Staphylococcus aureus*	< 100 ng/mL
Tularemia	*Francisella tularensis*	150,000-1 million CFU/mL
*Reported by manufacturer.		

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes (using optical reader), or user interprets presence/absence of a line.*
Unit weight: *(Optional optical readers).*
Guardian Reader™ – 4 lb; Defender TSR™ – 1.5 lb.
Power: *N/A; optional optical readers (rechargeable battery (25 test/charge) or AC).*
Cost: *Assay – $685/25 pack ($27 each); optional Guardian Reader™ – $7500; optional Defender TSR™ – $9995.*
Additional costs: Additional sample collection supplies. *Factory calibration is required every 5 years for either optical reader ($750).*
Assay shelf-life: *18 months from date of manufacture (ricin toxin assay); 24 months from date of manufacture (all others).*

Alexeter Technologies, LLC: RAID™

Phone: (877) 591-5571 *Manufacturer's website*: http://www.alexeter.com

Technology Summary

Alexeter RAID™ 5 and RAID™ 8 are multiplex LFA strips, which can test simultaneously for five and eight different agents, respectively, from a single sample. Each cartridge comes with a sampling tool that includes an integrated swab, buffer, and dropper dispenser. For 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). One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded.

The RAID™ TOX (a companion toxin assay to the RAID™ assays) was developed to address concerns about the hook effect that can result in false-negative results if too much analyte, particularly toxin, is present in the sample. If a negative result is obtained on the RAID™ 8 or RAID™ 5, a second, 50-fold diluted sample can be analyzed on the RAID™ TOX to address possible concerns about false-negative toxin results. The RAID™ TOX includes assays for SEB, ricin, and botulinum toxins. Additional internal control assays are included, showing what a positive and negative result should look like. These assays are not compatible with the Alexeter Defender™ TSR Reader or Guardian™ Reader.

Specifications

8-Agent (RAID™ 8), 5-Agent (RAID™ 5) and 5-Agent Toxin (RAID™ TOX) Immunoassay Cartridges

Biothreat Multi-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
8-Agent Assay (RAID™ 8)		
Tularemia	*Francisella tularensis*	1.6 million CFU/mL
Anthrax	*Bacillus anthracis*	100,000 spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	30 ng/mL
Brucellosis	*Brucella* species	1.5 million CFU/mL
Plague	*Yersinia pestis*	36,000 CFU/mL
Ricin toxin	*Ricinus communis*	6 ng/mL
Smallpox	Variola virus	1.6 million PFU/mL
SEB	*Staphylococcus aureus*	10 ng/mL
5-Agent Assay (RAID™ 5)		
Anthrax	*Bacillus anthracis*	100,000 spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	30 ng/mL
Plague	*Yersinia pestis*	36,000 CFU/mL
Ricin toxin	*Ricinus communis*	6 ng/mL
SEB	*Staphylococcus aureus*	10 ng/mL
3-Agent Assay (RAID™ TOX)		
Botulism (botulinum toxin)	*Clostridium botulinum*	30 ng/mL
Ricin toxin	*Ricinus communis*	6 ng/mL
SEB	*Staphylococcus aureus*	10 ng/mL
*Reported by manufacturer.		

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *User interprets presence/ absence of a line.*
Unit weight: *Negligible.*
Power: *N/A.*
Cost: *Assay – RAID™ 8 – $995/10 pack ($100 each; $12/agent);*
RAID™ 5 – $695/10 pack ($70 each; $14/agent);
RAID™ TOX –$495/10 pack (50 each; $17/agent).
Additional costs: *None.*
Assay shelf-life: *18 months from date of manufacture.*

Peer-Reviewed References

Slotved H. C., Sparding N., Tanassi J. T., Steenhard N. R., Heegaard N. H. Evaluating 6 ricin field detection assays. *Biosecurity and bioterrorism: biodefense strategy, practice, and science*. **2014**, Jul-Aug;12(4):186-9. DOI: 10.1089/bsp.2014.0015

This study compared eight commercially available ricin detection assays using pure ricin toxin. The eight assays compared are LFDs from AdVnt Biotechnologies (BADD™, Pro Strips™), Environics (ENVI Assay System), Alexeter Technologies (RAID™ 8, RAID™ DX, RAID™ TOX), Tetracore (BioThreat Alert®), BBI Detection (IMASS™). Ricin concentrations ranging from 0.625 to 20 ng/mL were tested with respect to manufacturers' stated detection limits. BADD™ and Pro Strips™ did not detect ricin at 20 ng/mL. The ENVI Assay System detected ricin at 20 ng/mL, gave mixed results at 10 ng/mL, and did not detect ricin at 5 ng/mL. The RAID™ 8, RAID™ DX and RAID™ TOX did not detect ricin at 20 ng/mL. The BioThreat Alert® detected ricin at 20, 10, and 5 ng/mL, but not at 2.5 or 1.5 ng/mL. The IMASS™ device detected ricin at 20 and 10 ng/mL but not at lower concentrations tested. The only assay evaluated, which was able to detect ricin at the detection limit stated by the manufacturer, was the BioThreat Alert®.

Slotved, H.-C.; Tanassi, J. T.; Sparding, N.; Lindqvist, A.; Steenhard, N. R.; Heegaard, N. H. H. Botulinum Toxin Field Assays Evaluated Using Cosmetic Botox Preparations**.** *Biosecurity and bioterrorism: biodefense strategy, practice, and science* **2013**, *11,* 280-286. DOI: 10.1089/bsp.2013.0050.

This study compared several different botulinum detection technologies using botulinum toxins (BoNTs) A and B, as well as four pharmaceutical-grade cosmetic botulinum toxin preparations. An ELISA method was used as a baseline and compared to an immunoaffinity column and LFAs from AdVnt Biotechnologies (1-agent BADD™ and 5-agent ProStrips), Environics (ENVI Assay system), and Alexeter (RAID DX kit, which contains an 8-agent RAID 8 LFA and a 3-agent RAID TOX LFA). Relatively low concentrations of BoNTs (both commercial and pharmaceutical sources) were used and were not detected by most of the LFAs. BADD™ did not detect pharmaceutical BoNT A (100 ng/mL), commercial BoNT A (50 ng/mL), or commercial BoNT B (500 ng/mL and 10,000 ng/mL). Pro Strips did not detect commercial BoNT A (50 ng/mL and 10,000 ng/mL), commercial BoNT B (10,000 ng/mL), or pharmaceutical BoNT A (100 ng/mL). The ENVI Assay System gave one positive result and one negative result with commercial BoNT A (10,000 ng/mL), but did not detect pharmaceutical BoNT A (100 ng/mL and 27.5 ng/mL). The RAID 8 and RAID TOX did not detect pharmaceutical BoNT A (27.5 ng/mL and 13.75 ng/mL), although those concentrations are below the manufacturer stated LOD for BoNT.

ANP Technologies®, Inc.: NIDS®

Phone: (302) 283-1730 *Manufacturer's website*: http://anptinc.com

Technology Summary

The Nano Intelligent Detection System (NIDS®) consists of multiplex LFA strips and an optional optical reader for detection. Sample collection supplies must be purchased separately.

NIDS® immunoassay strips are formatted for the detection of three, four, or five agents in a single cartridge. The manufacturer states 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 can produce false-negative results when very concentrated samples are analyzed.

After sample collection and solubilization, 5 drops (~100 µL) of sample are placed in the sample well of the test strip and the strip is allowed to develop for 15 minutes and placed into the optical reader for detection and automatic readout or it can be read manually. One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded. This product is also available from Smiths Detection.

The 3- and 4-agent assays are available for the general public, but the 5-agent assays are only

Specifications

Nano Intelligent Detection System (NIDS®) Immunoassay Cartridges, Sampling Kit and Optical Reader

Biothreat Multi-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD**
3-Agent Assay (NIDS® 3-Plex 3)		
Anthrax	*Bacillus anthracis*	Not reported
Plague	*Yersinia pestis*	Not reported
Tularemia	*Francisella tularensis*	Not reported
4-Agent Assay (NIDS® 4-Plex 5)		
Botulism (botulinum toxin A)	*Clostridium botulinum*	Not reported
Botulism (botulinum toxin B)	*Clostridium botulinum*	Not reported
Ricin toxin	*Ricinus communis*	Not reported
SEB	*Staphylococcus aureus*	Not reported
5-Agent Assay (NIDS® 5-Plex 1) – for US Govt Use Only		
Anthrax	*Bacillus anthracis*	Not reported
Ricin toxin	*Ricinus communis*	Not reported
Botulism (botulinum toxin)	*Clostridium botulinum*	Not reported
SEB	*Staphylococcus aureus*	Not reported
Tularemia	*Francisella tularensis*	Not reported
5-Agent Assay (NIDS® 5-Plex 2) – for US Govt Use Only		
Brucella	*Brucella melitensis*	Not reported
Smallpox	Variola virus	Not reported
Q fever	*Coxiella burnetii*	Not reported
Plague	*Yersinia pestis*	Not reported
Viral encephalitis	Venezuelan equine encephalitis	Not reported
5-Agent Assay (NIDS® 5-Plex 3) – for US Govt Use Only		
Brucella	*Brucella melitensis*	Not reported
Smallpox	Variola virus	Not reported
Q fever	*Coxiella burnetii*	Not reported
Plague	*Yersinia pestis*	Not reported
Cholera	*Vibrio cholerae*	Not reported

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes (using optical reader), or user interprets presence/absence of a line.*
Unit weight: *12 oz. (optical reader) and 10 lb (complete NIDS® kit).*
Power: *N/A; optional optical reader (3 AA batteries for 6-7 hours continuous operation).*
Cost: *Assay – $60-$80 for 3-4 agent cartridge; optional optical reader module – $6900; complete kit – $9000 (includes optical reader, sampling kits, and ten pairs of assay strips).*
Additional costs: Sample collection supplies.
Assay shelf-life: *24 months from date of manufacture.*

available to federal government organizations. Customized assays for *E. coli* O157:H7, *Salmonella*, and *Listeria* are also available.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system). This product (optical reader and immunoassay cartridges) has received a "Designated" classification (proven effectiveness, with confidence of repeatability) by DHS as part of its SAFETY Act of 2002 (www.safetyact.gov).

Peer-Reviewed References

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

BBI Detection, LLC: IMASS™

Phone: (888) 223-3269 *Manufacturer's website*: http://www.bbidetection.com

Technology Summary

BBI Detection has developed a multiplex LFA device with an integrated sampling system for surfaces, powders, or liquids. The device consists of a handheld cylinder that contains a sampling sponge at one end and eight lateral flow immunoassay strips integrated within the barrel of the cylinder. To initiate sampling, the user unscrews the end cap of the IMASS™ device and adds the contents of a buffer solution bottle to the IMASS™ sponge. The user then wipes the IMASS™ sponge over the surface to be sampled and replaces the cap, screwing down tightly to engage the driving nut, which initiates the sample flow onto the LFAs. The device is placed vertically on a flat surface while the assay develops. Results from the eight LFAs can be read manually in approximately 2 to 15 minutes.

One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system) and ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices).

Peer-Reviewed References

Slotved H. C., Sparding N., Tanassi J. T., Steenhard N. R., Heegaard N. H. Evaluating 6 ricin field detection assays. *Biosecurity and bioterrorism: biodefense strategy, practice, and science*. **2014**, Jul-Aug;12(4):186-9. DOI: 10.1089/bsp.2014.0015

This study compared eight commercially available ricin detection assays using pure ricin toxin. The eight assays compared are LFDs from AdVnt Biotechnologies (BADD™, Pro Strips™), Environics (ENVI Assay System), Alexeter Technologies (RAID™ 8, RAID™ DX, RAID™ TOX), Tetracore (BioThreat Alert®), BBI Detection (IMASS™). Ricin concentrations ranging from 0.625 to 20 ng/mL were tested with respect to manufacturers' stated detection limits. BADD™ and Pro Strips™ did not detect ricin at 20 ng/mL. The ENVI Assay System detected ricin at 20 ng/mL, gave mixed results at 10 ng/mL, and did not detect ricin at 5 ng/mL. The RAID™ 8, RAID™ DX

Specifications

IMASS™ 8-Agent Immunoassay Cartridge With Integral Sampling Device

Biothreat 8-Agent Assay		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	10,000 spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	1 ng/mL
Brucellosis	*Brucella* species	10,000 – 1 million CFU/mL
Glanders	*Burkholderia mallei*	1000 CFU/mL
Plague	*Yersinia pestis*	100 million CFU/mL
Ricin toxin	*Ricinus communis*	1 ng/mL
SEB	*Staphylococcus aureus*	1 ng/mL
Tularemia	*Francisella tularensis*	10,000 CFU/mL
*Reported by manufacturer		

Assay time: *~15 minutes.*
Required sample preparation? *No.*
Automatic results display? *User interprets presence/absence of a line.*
Unit weight: *Negligible.*
Power: *N/A.*
Cost: *Assay – $1270/10 pack ($127 each; $16/agent).*
Additional costs: *None.*
Assay shelf-life: *12 months from date of manufacture (at 4-28°C).*

and RAID™ TOX did not detect ricin at 20 ng/mL. The BioThreat Alert® detected ricin at 20, 10, and 5 ng/mL, but not at 2.5 or 1.5 ng/mL. The IMASS™ device detected ricin at 20 and 10 ng/mL but not at lower concentrations tested. The only assay evaluated, which was able to detect ricin at the detection limit stated by the manufacturer, was the BioThreat Alert®.

Bruker Daltronics: Portable Toxin Detector (pTD)

Phone: (978) 663-3660 *Manufacturer's website*: https://www.bruker.com/

Technology Summary

The pTD is a field-able platform for automated detection of biological toxins based on electrochemical ELISA. Antibodies immobilized on gold electrodes attached to a toxin "chipstick" capture corresponding toxins from an applied liquid or solubilized sample, which must be neutralized and filtered first. All buffers (i.e., sample buffer and neutralization buffer) and consumables necessary for sample preparation are included in the pTD Sample Preparation Kit. Detection and quantitation is based on the measured electrical current of an enzymatic redox reaction. The current correlates to the amount of target molecules captured by the antibodies.

The pTD Toxin Test Kit BWA I contains biochips immobilized with specific antibodies for the detection of toxins. Two independent electrode positions are used for detection of each target substance. In addition, three non-specifically coated negative control positions for deriving background current are measured on every chip as well as three 100% positive internal standards.

The pTD instrument utilizes a disposable assay kit, which can detect five toxins in parallel: Botulinum neurotoxin A, B, and E; Ricin; and staphylococcal enterotoxin B.

Peer-Reviewed References

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

Specifications

pTD Automated Electrochemical ELISA 5-Agent Toxin Detection System

Biothreat Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Toxin Assay BWA I		
Botulism (botulinum toxin A)	*Clostridium botulinum*	5 ng/mL
Botulism (botulinum toxin B)	*Clostridium botulinum*	5 ng/mL
Botulism (botulinum toxin E)	*Clostridium botulinum*	Not reported
Ricin	*Ricinus communis*	1 ng/mL
SEB	*Staphylococcus aureus*	0.3 ng/mL
Toxin Assay BWA II		
Botulism (botulinum toxin A)	*Clostridium botulinum*	5 ng/mL
Botulism (botulinum toxin B)	*Clostridium botulinum*	5 ng/mL
Botulism (botulinum toxin F)	*Clostridium botulinum*	10 ng/mL
Ricin	*Ricinus communis*	1 ng/mL
SEB	*Staphylococcus aureus*	0.3 ng/mL
*Reported by manufacturer.		

Assay time: *25 minutes.*
Required sample preparation? *Moderate.*
Automatic results display? *Yes.*
Unit weight: *31 lb.*
Power: *Battery, 24V DC (or 110-240 V AC/DC)*
Cost: *Assay – $1895/15 pack ($126 each; $25/agent); instrument – $69,130.*
Additional costs: *Laptop computer; Wash kit ($95); Sample preparation kit ($60/sample).*
Assay shelf-life: *6 months from date of manufacture (at 2-8 ºC).*

Environics, Inc.: ENVI Assay System Gold

Phone: (410) 612-1250 *Manufacturer's website*: http://www.environicsusa.com

Technology Summary

The Environics ENVI Assay System Gold are 1-agent LFAs that can be read manually or with an optical reader. The Environics Reader Module is designed to be an add-on module for the ChemPro®100 Chemical Detector, but can be used alone if it is connected to a PC. The optical reader can be powered by a USB port or directly from the ChemPro®100 Chemical Detector. Colorimetric ENVI assays include all materials required for analysis (e.g., sampling swabs, buffers, and transfer pipettes) packaged in a box that doubles as a sample-preparation platform.

One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system).

Peer-Reviewed References

Slotved H. C., Sparding N., Tanassi J. T., Steenhard N. R., Heegaard N. H. Evaluating 6 ricin field detection assays. *Biosecurity and bioterrorism: biodefense strategy, practice, and science*. **2014**, Jul-Aug;12(4):186-9. DOI: 10.1089/bsp.2014.0015

This study compared eight commercially available ricin detection assays using pure ricin toxin. The eight assays compared are LFDs from AdVnt Biotechnologies (BADD™, Pro Strips™), Environics (ENVI Assay System), Alexeter Technologies (RAID™ 8, RAID™ DX, RAID™ TOX), Tetracore (BioThreat Alert®), BBI Detection (IMASS™). Ricin concentrations ranging from 0.625 to 20 ng/mL were tested

Specifications

ENVI Assay System Gold: ChemPro®100 Chemical Detector, Optical Reader Module, and Immunoassay Cartridges

Biothreat 1-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Botulism (botulinum toxin)	*Clostridium botulinum*	10 ng/mL
Ricin toxin	*Ricinus communis*	5 ng/mL
SEB	*Staphylococcus aureus*	13.1 ng/mL
Anthrax	*Bacillus anthracis*	100,000 spores/mL
*Reported by manufacturer.		

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes (using optical reader), or user interprets presence/absence of a line.*
Unit weight: *ChemPro®100 and optical reader – 2.3 lb.*
Power: *N/A; optional ChemPro®100 and optical reader – rechargeable Li-ion batteries (8 hours).*
Cost: *Assay – $400-$650/10 pack ($40-$65 each); optional Bioassay Reader Module + PC software – $4500 (PC not included); ChemPro®100 (optional dual chemical/ biological reader can be used instead of Reader Module and PC) – $14,995.*
Additional costs: *None.*
Assay shelf-life: *12-24 months from date of manufacture.*

with respect to manufacturers' stated detection limits. BADD™ and Pro Strips™ did not detect ricin at 20 ng/mL. The ENVI Assay System detected ricin at 20 ng/mL, gave mixed results at 10 ng/mL, and did not detect ricin at 5 ng/mL. The RAID™ 8, RAID™ DX and RAID™ TOX did not detect ricin at 20 ng/mL. The BioThreat Alert® detected ricin at 20, 10, and 5 ng/mL, but not at 2.5 or 1.5 ng/mL. The IMASS™ device detected ricin at 20 and 10 ng/mL but not at lower concentrations tested. The only assay evaluated, which was able to detect ricin at the detection limit stated by the manufacturer, was the BioThreat Alert®.

Slotved, H.-C.; Tanassi, J. T.; Sparding, N.; Lindqvist, A.; Steenhard, N. R.; Heegaard, N. H. H. Botulinum Toxin Field Assays Evaluated Using Cosmetic Botox Preparations**.** *Biosecurity and bioterrorism: biodefense strategy, practice, and science* **2013**, *11,* 280-286. DOI: 10.1089/bsp.2013.0050.

This study compared several different botulinum detection technologies using botulinum toxins (BoNTs) A and B, as well as four pharmaceutical-grade cosmetic botulinum toxin preparations. An ELISA method was used as a baseline and compared to an immunoaffinity column and LFAs from AdVnt Biotechnologies (1-agent BADD™ and 5-agent Pro Strips), Environics (ENVI Assay system), and Alexeter (RAID DX kit, which contains an 8-agent RAID 8 LFA and a 3-agent RAID TOX LFA). Relatively low concentrations of BoNTs (both commercial and pharmaceutical sources) were used and were not detected by most of the LFAs. BADD™ did not detect pharmaceutical BoNT A (100 ng/mL), commercial BoNT A (50 ng/mL), or commercial BoNT B (500 ng/mL and 10,000 ng/mL). ProStrips did not detect commercial BoNT A (50 ng/mL and 10,000 ng/mL), commercial BoNT B (10,000 ng/mL), or pharmaceutical BoNT A (100 ng/mL). The ENVI Assay System gave one positive result and one negative result with commercial BoNT A (10,000 ng/mL), but did not detect pharmaceutical BoNT A (100 ng/mL and 27.5 ng/mL). The RAID 8 and RAID TOX did not detect pharmaceutical BoNT A (27.5 ng/mL and 13.75 ng/mL), although those concentrations are below the manufacturer stated LOD for BoNT.

GenPrime, Inc.: Toxin Screen

Phone: (866) 624-9855 *Manufacturer's website*: http://www.genprime.com

Technology Summary

This immunoassay toxin test is a multiplex LFA that contains assays to detect ricin, botulinum serotype A, and SEB in a single cartridge. The immunoassay is designed to work with the Prime Alert® DNA detection sample buffers, so both the DNA test and assay for toxins can be performed from a single solubilized sample. The kit includes buffer, a sample vial, a sampling scoop and a transfer pipette, as well as a claw sampler used for reaching into envelopes.

One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded. This product is also distributed through Smiths Detection.

The management system governing the manufacture of this product is ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices).

Peer-Reviewed References

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

Specifications

Toxin Screen 3-Agent Immunoassay Cartridge

Biothreat 3-Agent Assay		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Botulism (botulinum toxin)	*Clostridium botulinum*	400 ng/mL
Ricin toxin	*Ricinus communis*	400 ng/mL
SEB	*Staphylococcus aureus*	Not reported
*Reported by manufacturer.		

Assay time: *~10 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes, user interprets presence/absence of a line.*
Unit weight: *Negligible.*
Power: *N/A.*
Cost: *Assay – $100 ($33/agent).*
Additional costs: *None.*
Assay shelf-life: *12 months from date of manufacture.*

Menon Biosensors, Inc.: MENTOR-100 Biodetector

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

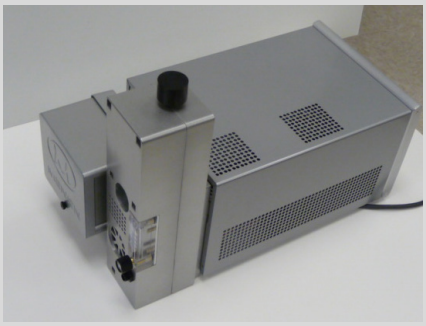
Technology Summary

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

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

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

Specifications



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

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

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

Peer-Reviewed References

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

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

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

Technology Summary

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

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

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

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

Specifications

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

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

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

Peer-Reviewed References

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

Meso Scale Defense™: PR2 1800

Phone: (240) 314-2795 *Manufacturer's website*: www.mesoscaledefense.com

Technology Summary

The Meso Scale Defense™ (MSD) PR2 1800 uses MSD's MULTI-ARRAY® electrochemiluminescence technology to multiplex immunoassays for biothreat agents in a 96-well plate format. MULTI-ARRAY® technology is a combination of electrochemiluminescence detection and patterned arrays. The detection of the biological material requires electrochemical stimulation of reporter molecules coupled to the binding antibody.

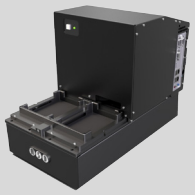
Each well of the plate has an array of up to 25 different antibodies to enable simultaneous measurement of multiple targets (including bacteria, viruses, toxins, and internal process controls). The PR2 1800 has been used to test a wide variety of sample types including many clinical matrices, dry filter unit extracts, aerosol samples, food and beverage samples, water, and soil samples.

To conduct an analysis, the user suspends the sample into reagents compatible with the instrument and introduces the liquid sample into the instrument. The instruments use custom-designed optics and photodetectors to collect and quantitatively measure light emitted from the microplates. Proprietary electronics and signal processing algorithms convert the measured signal into data output. For low density plates (i.e., fewer antibodies per well), results can be obtained in approximately 15 minutes, but results can require up to 1 hour for high-density plate formats.

Peer-Reviewed References

No peer-reviewed publications were found that evaluate the use of this product for the detection of biothreat agents. However, numerous peer-reviewed articles describe the performance of this system for the detection of clinical analytes and additional publications detail the chemistry and fundamentals of the system operation.

Specifications



PR2 1800 Multiplexed Electrochemiluminescent Immunoassay Detection System

Biothreat Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	1000 CFU/mL
Plague	*Yersinia pestis*	350 CFU/mL
Q fever	*Coxiella*	30,000 CFU/mL
Tularemia	*Francisella tularensis*	450 CFU/mL
Smallpox	Variola virus	300 PFU/mL
Hemorrhagic fever	Ebola virus	170 PFU/mL (Zaire)
Hemorrhagic fever	Marburg virus	250 PFU/mL (Musoki)
Hemorrhagic fever	Lassa virus	200 PFU/mL
Abrin Toxin	*Abrus precatorius*	150 pg/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	10 pg/mL
Ricin Toxin	*Ricinus communis*	5 pg/mL
SEB	*Staphylococcus aureus*	0.5 pg/mL
Shiga-like toxin 1	*Escherichia coli*	5 pg/mL
Shiga-like toxin 2	*Escherichia coli*	3 pg/mL
T-2 Toxin	Various fungi	4 ng/mL
Saxitoxin	Marine microorganisms	30 pg/mL
Malaria	*Plasmodium falciparum*	100 pg/mL for HRP-2
*Reported by manufacturer.		

Assay time: *15-60 minutes.*
Required sample preparation? *Moderate.*
Automatic results display? *Yes.*
Unit weight: *30 lb.*
Power: *120-240 VAC / 65 W average / 75 W peak.*
Cost: *Assays – $1-$4 each; instrument – $80,000.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *12 months from date of manufacture.*

NBC-SYS: KDTB® Gold

Phone: +33 4 77 19 19 27 *Manufacturer's website*: http://www.nexter-group.fr/nexter/Flipping_Book/Export_FR/#198

Technology Summary

The KDTB GOLD® kits contain 8 different LFAs for the detection of ricin; botulinum toxin A, B, E; staphylococcal enterotoxin B; anthrax; plague; and tularemia.

Sample collection and preparation supplies (e.g., dilution microtubes, plastic pipettes, sampling swabs, and dilution buffer) are provided.

Once the sample is solubilized, six drops are deposited on the test strip. In 15 minutes, the result is observed by eye or recorded with a small electronic reader for record keeping.

One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded.

Peer-Reviewed References

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

Specifications

KDTB Gold 1-Agent Immunoassays

Biothreat 1-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	2 million spores/mL
Plague	*Yersinia pestis*	2 million CFU/mL
Tularemia	*Francisella tularensis*	20,000 CFU/mL
Botulism (botulinum toxin A)	*Clostridium botulinum*	5 ng/mL
Botulism (botulinum toxin B)	*Clostridium botulinum*	5 ng/mL
Botulism (botulinum toxin E)	*Clostridium botulinum*	5 ng/mL
Ricin	*Ricinus communis*	1 ng/mL
SEB	*Staphylococcus aureus*	1 ng/mL
*Reported by manufacturer.		

Assay time: *15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes (using optional optical reader), or user interprets presence/absence of a line.*
Unit weight: *Negligible.*
Power: *N/A.*
Cost: *Assay kit – $2150/40 assays ($54/each); optional optical reader – $3225.*
Additional costs: *None.*
Assay shelf-life: *24 months from date of manufacture.*

New Horizons Diagnostics, Inc.: Smart™-II

Phone: (443) 543-5755 *Manufacturer's website*: http://www.nhdiag.com

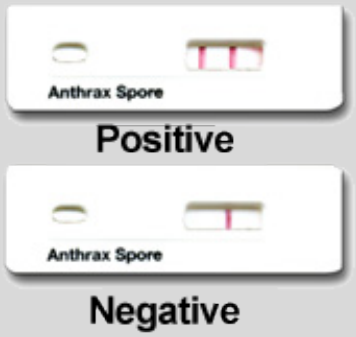
Technology Summary

The New Horizon Diagnostics Smart™-II is a standard LFA. Each device tests for one agent at a time. One kit contains the LFA device, plastic droppers, and "Chase" buffer. A collection kit (either New Horizon Diagnostics' kit or another compatible sample collection kit) must be purchased separately. If the sample contains visible debris or large particles, a sample processing kit should be used (also sold by New Horizon Diagnostics) 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 3 minutes for the sample to absorb in the sample well, two drops of Chase buffer are added from the Chase buffer dropper bottle. After a 15 minute incubation, the results can be read.

One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicate the agent was detected. The absence of a control line indicates the assay is invalid and any positive or negative test result must be disregarded.

The company offers a cholera assay that is Food and Drug Administration (FDA) approved. They also offer immunochromatographic assays for *E. coli* and *Salmonella*.

Specifications



Smart™-II 1-Agent Immunoassays

Biothreat 1-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	100,000 spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	10 ng/mL
Plague	*Yersinia pestis*	<10 ng/mL
Ricin toxin	*Ricinus communis*	10 ng/mL
SEB	*Staphylococcus aureus*	50 ng/mL
Tularemia	*Francisella tularensis*	1 million CFU/mL
*Reported by manufacturer.		

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes, user interprets presence/absence of a line.*
Unit weight: *Negligible.*
Power: *N/A.*
Cost: *Assay – $575/25 pack ($23 each).*
Additional costs: Sample collection supplies.
Assay shelf-life: *12 months from date of manufacture.*

Peer-Reviewed References

Zasada A.A.; Formińska, K.; Zacharczuk, K.; Jacob, D., and Grunow, R. "Comparison of Eleven Commercially Available Rapid Tests for Detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*." *Letters in Applied Microbiology*. **2015**, DOI: 10.1111/lam.12392.

This study compared 11 different commercially available rapid tests for detecting *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*. For each detection platform, the manufacturer's instructions were followed and tested against each of the aforementioned bacteria in concentrations ranging from 10^3 to 10^8 CFU/mL. *Y. pestis* was detected at ≥ 10^6 CFU/mL for the New Horizons Diagnostics SMART-II LFA, at ≥ 10^7 CFU/mL for the Tetracore BioThreat Alert LFA, and at ≥ 10^8 CFU/mL for the AdVnt BADD LFA. *Bacillus anthracis* was detected at ≥ 10^8 spores/mL for the New Horizons Diagnostics SMART-II LFA and the Tetracore BioThreat Alert LFA, but the AdVnt BADD LFA did not detect this

concentration. *F. tularensis* was detected at ≥ 10^7 CFU/mL for the New Horizons Diagnostics SMART-II LFA and at ≥ 10^8 CFU/mL for the Tetracore BioThreat Alert LFA.

Peckham, G. D.; Hew, B. E.; Waller, D. F.; Holdaway, C.; Jen, M. Amperometric Detection of *Bacillus anthracis* Spores: A Portable, Low-Cost Approach to the ELISA. *Int. J. Electrochem.* **2013**, *2013,* Article 803485. DOI: 10.1155/2013/803485. http://www.hindawi.com/journals/ijelc/2013/803485/

This investigation compared an antibody-based method using amperometric signal generation to ELISAs and LFAs for detecting *Bacillus anthracis* (Sterne strain) spores. Tetracore's ELISA assay kits were used. The LFAs included BADD™, Smart™-II, and BioThreat Alert®. At least 15 of each LFA were tested with spore concentrations of 10^4 CFU/mL to 5 x 10^6 CFU/mL. All LFAs detected 5 x 10^6 CFU/mL in greater than >90% of trials. Performance decreased with decreasing spore concentration (<80% for 10^6 CFU/mL and <60% for 5 x 10^5 CFU/mL). Smart™-II was the only immunoassay tested that detected samples with 10^5 CFU/mL, but this was achieved in <40% of trials. BioThreat Alert® was reported to be the easiest to interpret with a dark, highly contrasted test line. The BADD™ readout was reported to be very faint, even at high spore concentrations. ELISA had 100% positive detection for all spore concentrations, however each assay took over 6 hours to complete. Amperometry, which required just over 1 hour to complete, detected spores in >90% of trials at >10^5 CFU/mL; however, performance decreased with decreasing spore concentration (86% for 5 x 10^4 CFU/mL and 47% for 10^4 CFU/mL).

Sha, J.; Endsley, J. J.; Kirtley, M. L.; Foltz, S. M.; Huante, M. B.; Erova, T. E.; Kozlova, E. V.; Popov, V. L.; Yeager, L. A.; Zudina, I. V.; Motin, V. L.; Peterson, J. W.; DeBord, K. L.; Chopra, A. K. Characterization of an F1 Deletion Mutant of *Yersinia pestis* CO92, Pathogenic Role of F1 Antigen in Bubonic and Pneumonic Plague, and Evaluation of Sensitivity and Specificity of F1 Antigen Capture-Based Dipsticks. *J. Clin. Microbiol.* **2011**, *49,* 1708-1715. DOI. 10.1128/jcm.00064-11.

Two LFAs for the detection of *Yersinia pestis* were compared in this evaluation. BioThreat Alert® and Smart™-II performed similarly and were able to detect 10^5 to 5 x 10^5 CFU/mL of the bacteria and 0.5 µg/mL for purified antigen (i.e., the surface protein from the bacteria) in PBS or infected whole mouse blood. The authors note that their limits of detection were not as low as some other studies, which could be related to differences in the purity of the antigen or strains of *Yersinia pestis* used in the various studies.

Gessler, F.; Pagel-Wieder, S.; Avondet, M-A. Bohnel, H. Evaluation of Lateral Flow Assays for the Detection of Botulinum Neurotoxin Type A and Their Application in Laboratory Diagnosis of Botulism. *Diagn. Microbiol. Infect. Dis*. **2007**, *57*, 243–249. DOI: 10.1016/j.diagmicrobio.2006.07.017.

This study evaluated BioThreat Alert®, Smart™-II, BADD™, and RAMP® assays for their ability to detect botulinum neurotoxin in three forms: purified BoNT A, toxin complex, and toxin in the supernatant of a *Clostridium botulinum* culture. Only BADD™ and RAMP® detected purified BoNT A; LODs were 100 ng/mL for BADD™ and 50 ng/mL for RAMP®. All assays detected the BoNT A complex and SMART™-II and BioThreat Alert had greater sensitivity for the BoNT A complex (10 ng/mL) than BADD™ (100 ng/mL) and RAMP® (250 ng/mL). For the *Clostridium botulinum* culture samples, the concentration of active toxin was estimated using a mouse lethality assay. The lowest concentration of toxin in culture medium that the BioThreat Alert®, Smart™-II, and BADD™ could detect was

100 MLD/mL, while RAMP® could detect only 2500 MDL/mL. BADD™ gave a false-positive result for a culture medium that did not contain *Clostridium botulinum* or botulinum toxin.

King, D.; Luna, V.; Cannons, A.; Cattani, J.; Amuso, P. Performance Assessment of Three Commercial Assays for Direct Detection of *Bacillus anthracis* Spores. *J. Clin. Microbiol*. **2003**, *41*, 3454–3455. DOI:10.1128/JCM.41.7.3454-3455.2003.

This brief study by the Florida Department of Health Laboratory evaluated three immunoassay tests including BioThreat Alert®, Osborne Scientific's 1st generation BADD (Note: Osborne's biothreat product line was acquired by AdVnt in 2003 and the current assay is 3rd generation), and Smart™-II. The tests were evaluated for *Bacillus anthracis* (Pasteur strain) detection at quantities ranging from 10^2 to 10^6 spores (the concentration of the test samples and volume of sample applied were not reported). Only 2 to 8 samples were tested at each concentration. All test kits could detect *Bacillus anthracis* at 10^6 spores. BADD™ and Smart™-II could detect 10^5 spores; however, BioThreat Alert® detected 10^5 spores only once in eight separate assays. None of the assays could detect fewer than 10,000 spores. Tests were allowed to develop for 15 minutes, although positive results were apparent within 5 minutes. *Bacillus cereus* and *Bacillus thuringiensis* (non-threat near neighbors that could potentially result in a false-positive) were also tested twice for each test strip. No false positives were observed for the BADD™ or BioThreat Alert® tests; however, the Smart™-II tests yielded one false-positive result for *Bacillus thuringiensis*.

PathSensors, Inc.: CANARY® Zephyr

Phone: (443) 557-6150 *Manufacturer's website*: http:www.pathsensors.com

Technology Summary

The Cellular Analysis and Notification of Antigen Risks and Yields (CANARY®) platform is a cell-based immunoassay system. It uses specially designed cells (B cells, which are white blood cells) to sense and respond to defined biothreats. The B cells have been engineered to produce specific antibodies on their surface that bind the biothreat agent. Agent binding to the B cell surface triggers the release of light from a bioluminescent protein within the cell.

Following sample collection (sampling kit sold separately) the swab is placed in a tube containing the swab diluent and shaken to displace the sample from the swab. A 1-mL sample of the diluent is centrifuged for 2 minutes using the included micro-centrifuge and then the liquid is decanted. After the addition of 3 drops of assay buffer, the sample is centrifuged again for 2 minutes. The 'biosensor' solution (containing engineered B cells) is then added to the sample tube and centrifuged for 5 seconds. The result is then determined by placing the sample tube into the luminometer. A 10-minute bead capture step is also needed for toxin targets. Detection is sensitive and fast; following sample collection, as few as 100 CFU/PFU of pathogen or picogram levels of toxin can be detected in 5 minutes. The LOD is ultimately determined by the affinity of the agent-specific antibody.

Assays are also available for *Salmonella*, *Listeria,* and *Ralstonia* species. The CANARY® Zephyr platform is currently in use at some USDA, FDA, and Department of Defense locations, as well as some State Health Departments. In addition, limited performance testing has been performed by Battelle in Columbus, Ohio.

Zephyr now offers a rackmounted configuration of the device that is designed for mobile lab use.

Specifications

CANARY® Zephyr B Cell-Based Immunoassay System

Biothreat 1-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	100-500 spores/mL
Ricin toxin	*Ricinus communis*	500 pg/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	160 pg/mL
Plague	*Yersinia pestis*	100-1000 CFU/mL
Smallpox	Variola virus	<500 pfu/mL
Tularemia	*Francisella tularensis*	100 CFU/mL
Ralstonia	*Ralstonia* species	10-100 CFU/mL
*Reported by manufacturer.		

Assay time: *5 minutes for bacteria and viruses. <15 minutes for toxins.*
Required sample preparation? *Moderate.*
Automatic results display? *Yes.*
Unit weight: *50 lb.*
Power: *110-220 VAC.*
Cost: *Assay – $80/5 pack ($16 each); instrument – $23,500.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *1 month at 4 °C or ≥1 year from date of manufacture in liquid nitrogen Dewar container.*

Peer-Reviewed References

Rider, T. H.; Petrovick, M. S.; Nargi, F. E.; Harper, J. D.; Schwoebel, E. D.; Mathews, R. H.; Blanchard, D. J.; Bortolin, L. T.; Young, A. M.; Chen, J.; Hollis, M. A. A B Cell-Based Sensor for Rapid Identification of Pathogens. *Science* **2003**, *301*, 213-215. DOI:10.1126/science.1084920.

This report describes the fundamental principles of the CANARY® detection approach used in the Zephyr system. First published in 2003, the detection approach remains fundamentally unchanged; however, the instrument platform (i.e., the Zephyr) has evolved into a system that is suitable for certain field deployments (e.g., mobile laboratories), and the assay sensitivity has improved. The detection principle utilizes B cells (lymphocytes) that have been engineered to express a calcium-sensitive bioluminescent protein that emits light when exposed to specific bacteria and viruses. The B cells contain membrane-bound antibodies to enable specific biothreat recognition. Upon cross-linking of the antibodies by specific bacteria or viruses, a rapid elevation of intracellular calcium concentration in the B cells occurs, resulting in the emission of light that is detected by a luminometer.

Research International, Inc.: RAPTOR™

Phone: (360) 805-4930 *Manufacturer's website*: http://www.resrchintl.com

Technology Summary

The RAPTOR™ is a portable automated 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 assay coupons. The RAPTOR™ is an automated sample processing system and fluorescence optical reader with integrated fluidics, electronics, and software, which uses proprietary disposable optical waveguide coupons. The system is highly configurable and users can define multi-step assay protocols and develop their own sample processing and assay protocols and assay coupons. There are also a range of pre-defined assay protocols and coupons for biothreat agents. Sample collection supplies must be purchased separately.

The disposable assay coupons are about the size of a credit card and contain four reaction surfaces (i.e., channels containing sensor elements/waveguides) that can be used to simultaneously detect four different biothreat agents. 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 agent 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 agent, 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 assay coupon, inserts the assay coupon into the automated system, 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

Specifications

RAPTOR™ Automated 4-Agent Immunoassay System

Available Biothreat Assays (pick 4)		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Ricin toxin	*Ricinus communis*	1 ng/mL
SEB	*Staphylococcus aureus*	0.1 – 0.5 ng/mL
Anthrax	*Bacillus anthracis* (vegetative Sterne cells)	100 CFU/mL
Anthrax	*Bacillus anthracis* (irradiated Ames spores)	50,000 CFU/mL
Bacterial infection	*E. coli* O157:H7	100 – 1000 CFU/mL
Protozoan infection	*Giardia lamblia*	50,000 CFU/mL
Plague	*Yersinia pestis* (F1 antigen target)	1 ng/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	1 – 10 ng/mL
Cholera toxin	*Vibrio cholerae*	0.1 – 1 ng/mL
Brucellosis	*Brucella abortus*	70,000 CFU/mL
Tularemia	*Francisella tularensis*	50,000 CFU/mL
Salmonellosis	*Salmonella typhimurium*	20,000 CFU/mL
Smallpox	Variola virus	10,000 PFU/mL
*Reported by peer-reviewed publications.		

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes.*
Unit weight: *12.3 lb (14.5 lb with battery).*
Power: *Optional military battery BA5590 (9-24 hours); rechargeable is also available.*
Cost: *Assay – $2000/10 pack ($200 each; $50/agent); automated system – $50,000.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *6 months from date of manufacture.*

blank run, takes 10 to 15 minutes to complete. If an assay is negative, the coupon can be reused in an 8- to 12-hour period for up to 30 assays or until a positive result is obtained.

Peer-Reviewed References

Kim, G-Y.; Morgan, M. T.; Ess, D.; Hahm, B-K; Kothapalli, A.; Valadez, A.; Bhunia, A. Detection of *Listeria monocytogenes* Using an Automated Fiber Optic Biosensor: RAPTOR. *Key Eng. Mater*. **2006**, 321-323, 1168-1171. DOI: 10.4028/www.scientific.net/KEM.321-323.1168.

This report by researchers at Purdue University and the Korean Institute of Agricultural Engineering describes the development of RAPTOR™ for the detection of *Listeria monocytogenes*. Pre-incubating the sample with the detection antibody for 30 minutes increased the sensitivity of the assay. The sensitivity of the assay for *Listeria monocytogenes* cultured 20 hours from a spiked hotdog sample was 5.4×10^7 CFU/mL.

Viswaprakash, N.; Kim, G.; Morgan, M. T.; Ess, D.; Hahm, B-K.; Kothapalli, S.; Valadez, A.; Geng, T.; Bhunia, A. K. Antibody Immobilization on Waveguides Using a Flow-Through System Shows Improved *Listeria monocytogenes* Detection in an Automated Fiber Optic Biosensor: RAPTOR™. *Sensors* **2006**, *6*, 808-822. DOI: 10.3390/s6080808. http://www.mdpi.com/1424-8220/6/8/808/pdf (accessed Feb 25, 2014).

This report by researchers at Purdue University and the Korean Institute of Agricultural Engineering describes the development of RAPTOR™ for the detection of *Listeria monocytogenes.* The developed sandwich immunoassay used a polyclonal antibody to capture *Listeria monocytogenes* from the sample and a monoclonal antibody to detect the captured *Listeria monocytogenes*. The optimized assay could detect 10^3 CFU/mL *Listeria monocytogenes* in PBS. In samples cultured from frankfurters spiked with *Listeria monocytogenes*, 5×10^5 CFU/mL *Listeria monocytogenes* could be detected. Negative control cultures of *Listeria rhamnosus* and *Enterococcus faecalis* also showed increasing signal with increasing concentration; however, they were at a lower level than the comparative signals with *Listeria monocytogenes.* Thus, samples containing relatively high concentrations of *Listeria rhamnosus* or *Enterococcus faecalis* could be interpreted as containing a low concentration of *Listeria monocytogenes*.

Jung, C. C.; Saaski, E. W.; McCrae, D. A.; Lingafelt, B. M.; Anderson, G. P. RAPTOR: A Fluoroimmunoassay-Based Fiber Optic Sensor for Detection of Biological Threat. *IEEE Sens. J*. **2003**, *3*, 352-360. DOI: 10.1109/JSEN.2003.815775.

This report by researchers at Research International, George Mason University, and the U.S. Naval Research Laboratory describes the RAPTOR™ in detail and summarizes improvements in hardware and assay chemistry that result in approximately a log-order improvement over the previous system. This study reported increases in assay signals for each improvement, and the authors state this translated to improvements in the LOD for the RAPTOR™; however, LOD results are not provided to support this statement. The report also briefly summarized an unpublished trial of the system conducted in June 2002 in which 17 of 17 samples containing 10^5 spores/mL *Bacillus anthracis* were detected when interspersed between 132 negative samples. In a similar trial in April 2001, the system's predecessor only detected 1 of 16 samples containing 10^5 spores/mL *Bacillus anthracis* interspersed between 256 negative samples.

Anderson, G. P.; Nerurkar, N. L. Improved Fluoroimmunoassays Using the Dye Alexa Fluor 647 with the RAPTOR, a Fiber Optic Biosensor. *J. Immunol. Methods*, **2002**, *271*, 17-24. DOI: 10.1016/S0022-1759(02)00327-7.

This report by researchers at the U.S. Naval Research Laboratory investigates the performance of the fluorescent dye Alexa Fluor 647 compared to Cy5 when used in immunoassays on the RAPTOR™ system. In both a direct binding immunoassay for ricin and a sandwich assay for staphylococcal enterotoxin B, the Alexa Fluor 647-labeled antibodies produced higher fluorescence signals than the Cy5-labeled antibodies. Higher signal intensity should translate to assays with increased sensitivities; however, this was not demonstrated in the scope of this study.

Response Biomedical Corp.: RAMP®

Phone: (888) 591-5577 *Manufacturer's website*: www.responsebio.com

Technology Summary

The Rapid Analyte Measurement Platform (RAMP®) is a fluorescence-based LFA system. The test uses a fluorescence-based detection scheme that requires an optical reader. Each RAMP® test kit includes 25 test cartridges with test tips, 25 sample vials, 25 powder-sampling microbrushes, 10 liquid sampling swabs, 1 transfer device, a marker pen, and an instruction card. The optical reader includes a waterproof Pelican™ case and an integral printer. Four test kits are available. Sample preparation for this test is slightly more involved than a standard colorimetric LFA; however, the test is more sensitive than standard LFAs. Initial swab sampling follows standard methods (i.e., swab and solubilize in a sample buffer). However, transferring the sample to the assay cartridge involves first mixing it 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 test proceeds like a typical LFA (i.e., add sample to sample window, insert into the fluorescence optical reader, and incubate for 15 minutes). 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. 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.

Specifications

RAMP® Optical Reader and 1-Agent Immunoassay Cartridges

Biothreat 1-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	620,000 spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	50 ng/mL
Ricin toxin	*Ricinus communis*	100 ng/mL
Smallpox	Variola virus	36 ng/mL
*Reported by manufacturer and peer-reviewed publications.		

Assay time: *~20 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes.*
Unit weight: *4.6 lb (20 lb including Pelican™ case and printer).*
Power: *Battery (100 tests) or AC.*
Cost: *Assay – Anthrax and smallpox $675/25 pack ($27 each), ricin and botulinum $599/25 pack ($24 each); required optical reader – $7000.*
Additional costs: *None.*
Assay shelf-life: *12 months from date of manufacture.*

The RAMP® anthrax test is the first commercial handheld anthrax test that has a "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."

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system) and ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices).

Peer-Reviewed References

Gessler, F.; Pagel-Wieder, S.; Avondet, M-A. Bohnel, H. Evaluation of Lateral Flow Assays for the Detection of Botulinum Neurotoxin Type A and Their Application in Laboratory Diagnosis of Botulism. *Diagn. Microbiol. Infect. Dis*. **2007**, *57*, 243–249. DOI: 10.1016/j.diagmicrobio.2006.07.017.

This study evaluated BioThreat Alert®, Smart™-II, BADD™, and RAMP® assays for their ability to detect botulinum neurotoxin in three forms: purified BoNT A, toxin complex, and toxin in the supernatant of a *Clostridium botulinum* culture. Only BADD™ and RAMP® detected purified BoNT A; LODs were 100 ng/mL for BADD™ and 50 ng/mL for RAMP®. All assays detected the BoNT A complex and SMART™-II and BioThreat Alert had greater sensitivity for the BoNT A complex (10 ng/mL) than BADD™ (100 ng/mL) and RAMP® (250 ng/mL). For the *Clostridium botulinum* culture samples, the concentration of active toxin was estimated using a mouse lethality assay. The lowest concentration of toxin in culture medium that the BioThreat Alert®, Smart™-II, and BADD™ could detect was 100 MLD/mL, while RAMP® could detect only 2500 MDL/mL. BADD™ gave a false-positive result for a culture medium that did not contain *Clostridium botulinum* or botulinum toxin.

Hoile, R.; Yuen, M.; James, G.; Gilbert, G. L. Evaluation of the Rapid Analyte Measurement Platform (RAMP®) for the Detection of Bacillus anthracis at a Crime Scene**.** *Forensic Sci. Int.* **2007**, *171*, 1-4. DOI: 10.1016/j.forsciint.2006.09.004.

This study investigated the accuracy and reliability of RAMP® for anthrax detection. To determine sensitivity, a clinical isolate of *Bacillus anthracis* was measured at six concentrations and detected in all three samples at 6.2 x 10^5 spores/mL (and higher concentrations) and in one of three samples at 5.1 x 10^5 spores/mL. Concentrations <4.8 x 10^5 spores/mL were not detected. *Bacillus anthracis* vegetative cells were detected at 10^8 CFU/mL, but lower concentrations were not analyzed. Specificity was determined by measuring *Bacillus subtilis*, *Bacillus thuringiensis,* and *Bacillus cereus* spores at concentrations ranging from 10^4 to 10^{10} spores/mL in duplicate with no false-positive results. A total of 11 household powders were also tested in triplicate at a concentration of 0.1 g/L with no false-positive results.

Harper, B. and Robinson, M. Method Modification (2004.08) to Field-Testing of Visible Powders on a Variety of Nonporous Environmental Surfaces: Field Study**.** *J. AOAC Int.* **2006**, *89*, 1622-1628. http://64.207.184.223/uploads/publications/Anthrax_-_Harper__Robinson_(Dugway)_2006.pdf (accessed Feb 28, 2014).

This paper summarizes RAMP® anthrax field-testing (conducted in a trailer) performed by six teams of first responders and civil support teams in Class C personal protective equipment. Each team consisted of 3 people with all but 1 team rotating the roles of team members among sampler, facilitator, and RAMP® operator, resulting in 14 different team members operating the RAMP®. *Bacillus anthracis* (Sterne strain) and *Bacillus thuringiensis* (Kurstaki) visible powder samples were collected from seven nonporous surfaces (i.e., plastic, stainless steel, ceramic tile, wood, rubber, sealed concrete, and food-grade painted wood) and solubilized/processed according to the RAMP® test instructions. A total of 1008 samples were analyzed. Eight incidences of errors or invalid results occurred, but sampling and analysis were repeated with the correct results. A total of 840 *Bacillus anthracis* samples were analyzed, resulting in 831 true positive results and 9 false-negative results. A total of 168 *Bacillus thuringiensis* samples were analyzed resulting in 165 true negative results and 3 false-positive results. Because the study included sampling, and sample processing and detection, the unexpected results and errors could be due to sampling or procedural/processing errors and not necessarily assay or instrument errors.

Stephenson, J. RAMP® Anthrax Test Cartridge. *J. AOAC Int.* **2005**, *88*, 202-203.

This article summarizes the protocol for using RAMP® for presumptive laboratory detection of *Bacillus anthracis* spores in environmental samples. No test data are reported in this paper.

Tetracore, Inc.: BioThreat Alert® Reader MX

Phone: (240) 268-5400 *Manufacturer's website*: http://www.tetracore.com

Technology Summary

Tetracore BioThreat Alert® Test Strips are standard 1-agent LFAs. Each BioThreat Alert® kit (containing 25 test strips) comes with 5 sample collection swabs, 5 sample vials, and 12 mL of buffer. 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 manually in the test window.

One line in the control zone indicates the agent was not detected, two lines (one in the control zone and one in the test zone) indicates the agent was detected. The absence of a control line indicates the assay is invalid, and any positive or negative test result must be disregarded.

For increased accuracy and sensitivity, an optional optical reader is available. The optical reader, which includes an optional attached stylus, is essentially a tablet-sized, handheld touchscreen device that provides an objective interpretation of the test results. Following the test strip incubation period, it takes approximately 20 seconds for the optical reader to analyze a test strip and give an output of positive, negative, or inconclusive. The optical reader also can save and print the test results, and it includes Bluetooth and Wi-Fi capabilities

Specifications

BioThreat Alert® Reader and 1-Agent Immunoassays

Biothreat 1-Agent Assays		
Disease/ Toxin	**Causative Agent/Source**	**LOD***
Abrin Toxin	*Abrus precatorius*	10-20 ng/mL
Anthrax	*Bacillus anthracis*	10,000-1 million CFU/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	5-20 ng/mL (BoNT A) 25-50 ng/mL (BoNT B)
Brucellosis	*Brucella* species	1-2 µg/mL
Plague	*Yersinia pestis*	100,000-1 million CFU/mL
Ricin toxin	*Ricinus communis*	2-5 ng/mL
SEB	*Staphylococcus aureus*	5-10 ng/mL
Smallpox	Variola virus	40 million-100 million CFU/mL
Tularemia	*Francisella tularensis*	30,000-400,000 CFU/mL
*Reported by manufacturer and peer-reviewed publications.		

Assay time: *~15 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes (using optical reader), or user interprets presence/absence of a line.*
Unit weight: *Reader – 3 lb.*
Power: *N/A; optional optical reader has rechargeable battery (6 hours).*
Cost: *Assay – $605/25 pack ($24 each); optional optical reader – $5500.*
Additional costs: *None.*
Assay shelf-life: *2 years for abrin and ricin toxin assays, 3 years for all others from date of manufacture.*

Peer-Reviewed References

Zasada A.A.; Formińska, K.; Zacharczuk, K.; Jacob, D., and Grunow, R. "Comparison of Eleven Commercially Available Rapid Tests for Detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*." *Letters in Applied Microbiology*. **2015**, DOI: 10.1111/lam.12392.

This study compared 11 different commercially available rapid tests for detecting *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*. For each detection platform, the manufacturer's instructions were followed and tested against each of the aforementioned bacteria in concentrations ranging from 10^3 to 10^8 CFU/mL. *Y. pestis* was detected at ≥ 10^6 CFU/mL for the New Horizons Diagnostics SMART-II LFA, at ≥ 10^7 CFU/mL for the Tetracore

BioThreat Alert LFA, and at ≥ 10^8 CFU/mL for the AdVnt BADD LFA. *Bacillus anthracis* was detected at ≥ 10^8 spores/mL for the New Horizons Diagnostics SMART-II LFA and the Tetracore BioThreat Alert LFA, but the AdVnt BADD LFA did not detect this concentration. *F. tularensis* was detected at ≥ 10^7 CFU/mL for the New Horizons Diagnostics SMART-II LFA and at ≥ 10^8 CFU/mL for the Tetracore BioThreat Alert LFA.

Slotved H. C., Sparding N., Tanassi J. T., Steenhard N. R., Heegaard N. H. Evaluating 6 ricin field detection assays. *Biosecurity and bioterrorism: biodefense strategy, practice, and science*. **2014**, Jul-Aug;12(4):186-9. DOI: 10.1089/bsp.2014.0015

This study compared eight commercially available ricin detection assays using pure ricin toxin. The eight assays compared are LFDs from AdVnt Biotechnologies (BADD™, Pro Strips™), Environics (ENVI Assay System), Alexeter Technologies (RAID™ 8, RAID™ DX, RAID™ TOX), Tetracore (BioThreat Alert®), BBI Detection (IMASS™). Ricin concentrations ranging from 0.625 to 20 ng/mL were tested with respect to manufacturers' stated detection limits. BADD™ and Pro Strips™ did not detect ricin at 20 ng/mL. The ENVI Assay System detected ricin at 20 ng/mL, gave mixed results at 10 ng/mL, and did not detect ricin at 5 ng/mL. The RAID™ 8, RAID™ DX and RAID™ TOX did not detect ricin at 20 ng/mL. The BioThreat Alert® detected ricin at 20, 10, and 5 ng/mL, but not at 2.5 or 1.5 ng/mL. The IMASS™ device detected ricin at 20 and 10 ng/mL but not at lower concentrations tested. The only assay evaluated, which was able to detect ricin at the detection limit stated by the manufacturer, was the BioThreat Alert®.

Ramage, J. G.; Prentice, K. W.; Marse, S. A.; Carter, A. J. Datta, S.; Drumgoole, R.; Gargis, S. R.; Griffin-Thomas, L.; Hastings, R.; Masri, H. P.; Reed, M. S.; Sharma, S. K.; Singh, A. K.; Swaney, E.; Swanson, T.; Gauthier, C.; Toney, D.; Pohl, J.; Shakamuri, P.; Stuchlik, O.; Elder, I. A.; Estacio, P. L.; Garber, E. A. E.; Hojvat, S.; Kellogg, R. B.; Kovacs, G.; Stanker, L.; Weigel, L.; Hodge, D. R.; Pillai, S. P. Comprehensive Laboratory Evaluation of a Specific Lateral Flow Assay for the Presumptive Identification of Abrin in Suspicious White Powders and Environmental Samples. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science.* **2014**, *12*, 49-62. DOI: 10.1089/bsp.2013.0080.

A comprehensive laboratory evaluation of BioThreat Alert® abrin test strips was conducted at five test sites to assess the immunoassay sensitivity, specificity, reproducibility, and limitations. Tests were conducted using 150 µL of sample and read both visually and with the BioThreat Alert® Reader. A total of 156 negative controls and 40 positive controls were run at the test sites during the course of the study with all samples producing expected results. Repeatability was assessed by measuring 240 abrin samples (120 at 25 ng/mL and 120 at 50 ng/mL) performed by 10 operators at the 5 sites on at least 2 different days. All visual readings resulted in a true positive result. Optical reader values for the abrin samples at 25 ng/mL (436 ± 95) and 50 ng/mL (698 ± 168) were significantly different. An inclusivity panel was prepared from the seeds of 11 cultivars of *Abrus precatorius* and tested at a final protein concentration of 1 µg/mL. All results were positive. Another panel was created from the seeds or leaves of 35 near neighbors of *Abrus precatorius* at a concentration of 10 µg/mL. All of the near-neighbor samples gave negative results, except one. *Abrus laevigatus* gave a false-positive result at all five test sites. A panel of 65 lectins was also tested at 5 µg/mL. All results were negative. A toxin/protein panel consisting of 11 proteins at 1 µg/mL was also tested for the potential to generate false-positive results. Only ricin A and B chain proteins, which by themselves are not health threats, gave false-positive results, but not other forms of ricin. It was determined that these false-positive results could be eliminated by the addition of

powdered milk to the sample buffer, although this caused a reduction in assay sensitivity. Abrin agglutinin (APA-1), which is also present in *Abrus precatorius* seeds but has ~250-fold lower toxicity than abrin, also gave a positive result. A white powder panel, consisting of 26 white powders commonly encountered in the field by first responders, was also tested. Powders were vortex-mixed in buffer (concentration not stated), allowed to settle for 5 minutes, and the supernatant was analyzed. A total of 18 of the powder suspensions produced a clear supernatant after settling, while 8 suspensions remained opaque. After the 15-minute assay development time, none of the white powders were found to interfere with the development of the positive control line and none gave a positive result for the presence of abrin. The powder suspensions were then tested after spiking (spiking volume not stated) with *Abrus precatorius* seed extract (10 µg/mL). All but one assay produced a positive sample result at all five test sites. Most of the powder did give reduced readings on the optical reader, with powdered toothpaste causing the greatest reduction and two of the false-negative results at two sites. BioWatch filter extracts were also analyzed at each site, including filter extracts spiked with *Abrus precatorius* bean extracts at approximately 10 µg/mL. Filter extracts did not affect the performance of the assay. The LOD for this abrin immunoassay was estimated to be 10 ng/mL.

Hodge, D. R.; Prentice, K. W.; Ramage, J. G.; Prezioso, S.; Gauthier, D.; Swanson, T. ; Hastings, R.; Basavanna, U.; Datta, S.; Sharma, S. K.; Garber, E. A. E.; Staab, A.; Pettit, D.; Drumgoole, R.; Swaney, E.; Estacio, P. L.; Elder, I. A.; Kovacs, G.; Morse, B. S.; Kellogg, R. B.; Stanker, L.; Morse, S. A.; Pillai, S. P. Comprehensive Laboratory Evaluation of a Highly Specific Lateral Flow Assay for the Presumptive Identification of Ricin in Suspicious White Powders and Environmental Samples. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science.* **2013,** *11,* 237-250. DOI: 10.1089/bsp.2013.0053.

This comprehensive study of BioThreat Alert® ricin test strips and the optical reader included evaluations of sensitivity, specificity, and reproducibility using panels of different sample types at five laboratories. Tests were conducted using 150 µL of sample and read both visually and with the BioThreat Alert® Reader. A total of 129 negative controls were analyzed during the course of the study. All negative control results yielded negative results as expected. Repeatability studies were conducted using ricin samples prepared at 20 and 40 ng/mL (120 samples analyzed at each concentration). All 240 samples analyzed by 9 different operators at 5 different sites, tested on at least 2 different days were correctly identified as containing ricin, and the optical reader values for samples of 20 ng/mL (681 ± 201) and 40 ng/mL (1191 ± 264) were significantly different. It should be noted that the optical reader values had some dependency on the time of the reading (e.g., 15 vs. 20 vs. 30 minutes), and the rate of change of optical reader value had some dependence on ricin concentration, although this has little consequence when the assay is used in a qualitative manner. With additional analysis of ricin samples for a LOD study, the sensitivity (LOD) of the assay was determined to be 3.6 ng/mL. For inclusivity testing (i.e., ability to correctly detect ricin from different plant cultivars), 18 different ricin cultivars were used to prepare samples at 667 ng/mL and tested once per site. All results were positive, although the authors point out in a detailed discussion that it is not possible to determine if the positive results are due solely to the presence of ricin. A series of different forms of ricin such as RCA_{60} and RCA_{120} (i.e., an "informational panel") was prepared at 667 ng/mL and tested once per site. As with most assays, this assay cannot discriminate among RCA_{60}, RCA_{120}, or ricin A chain. This comprehensive study includes a detailed discussion of results, most of which were as expected. A lectin panel (35 different lectins), which could cause false-positive results by interfering with the assay antibodies, was prepared at 667 ng/mL and tested once per site (175 total samples). No false positives were observed and in no instances did the lectins

cause a failure of the positive control line to appear. A near-neighbor panel (to examine additional potential false positives) consisted of crude extracts prepared from the seeds or leaves of near neighbors of *Ricinus communis* and *Abrus precatorius* at 6.67 µg/mL, and the samples were tested once per site. Not all potential near neighbors were tested because many are not commercially available and are not common to the United States. For those near neighbors tested, all sample lines resulted in a negative result and all control lines were positive. A white powder panel included 24 powders commonly encountered by first responders and the LRN, which were prepared at 10 mg/mL, vortex-mixed in buffer, allowed to settle for 5 minutes, followed by analysis of the supernatant. A total of 18 of the powder suspensions produced a clear supernatant after settling, while 6 suspensions remained opaque. After the 15 minute assay development time, none of the white powders were found to interfere with the development of the positive control line, and none gave a positive result for the presence of ricin. The 24 powder suspensions were then tested at each site after spiking with castor bean extract (containing approximately 1% ricin toxin), with a final protein concentration of approximately 66.7 ng/mL per sample. All of the assays produced a positive sample result for the ricin-spiked powder suspensions and no interference occurred with the development of the positive control line, although most of the powders did give reduced readings on the optical reader. BioWatch filter extracts were also analyzed at each site, including filter extracts spiked with castor bean extracts at approximately 6.67 µg/mL. Filter extracts did not affect the performance of the assay.

Peckham, G. D.; Hew, B. E.; Waller, D. F.; Holdaway, C.; Jen, M. Amperometric Detection of *Bacillus anthracis* Spores: A Portable, Low-Cost Approach to the ELISA. *Int. J. Electrochem.* **2013**, *2013,* Article 803485. DOI: 10.1155/2013/803485. http://www.hindawi.com/journals/ijelc/2013/803485/

This investigation compared an antibody-based method using amperometric signal generation to ELISAs and LFAs for detecting *Bacillus anthracis* (Sterne strain) spores. Tetracore's ELISA assay kits were used. The LFAs included BADD™, Smart™-II, and BioThreat Alert®. At least 15 of each LFA were tested with spore concentrations of 10^4 CFU/mL to 5 x 10^6 CFU/mL. All LFAs detected 5 x 10^6 CFU/mL in greater than >90% of trials. Performance decreased with decreasing spore concentration (<80% for 10^6 CFU/mL and <60% for 5 x 10^5 CFU/mL). Smart™-II was the only immunoassay tested that detected samples with 10^5 CFU/mL, but this was achieved in <40% of trials. BioThreat Alert® was reported to be the easiest to interpret with a dark, highly contrasted test line. The BADD™ readout was reported to be very faint, even at high spore concentrations. ELISA had 100% positive detection for all spore concentrations, however each assay took over 6 hours to complete. Amperometry, which required just over 1 hour to complete, detected spores in >90% of trials at >10^5 CFU/mL; however, performance decreased with decreasing spore concentration (86% for 5 x 10^4 CFU/mL and 47% for 10^4 CFU/mL).

Townsend, M. B.; MacNeil, A.; Reynolds, M. G.; Hughes, C. M.; Olson, V. A.; Damon, I. K.; Karem, K. L. Evaluation of the Tetracore Orthopox BioThreat® Antigen Detection Assay Using Laboratory Grown Orthopoxviruses and Rash Illness Clinical Specimens. *J. Virol. Methods* **2013**, *187*, 37- 42. DOI: 10.1016/j.jviromet.2012.08.023.

This study evaluated Tetracore's BioThreat® Alert assay for orthopoxvirus, which is an assay designed to detect the causative agent of smallpox. Using a 150 µL sample volume, assay results were read after 15 minutes both visually and using an optical lateral flow reader from Qiagen. Cultured Congo Basin strains of Vaccinia virus and Monkeypox virus at concentrations of 10^4 to 10^8 PFU/mL were used to evaluate the sensitivity of the assay in duplicate by two different users. The BioThreat® Alert assay

positively detected all samples at concentrations of 10^7 PFU/mL for both viruses and 4 of 7 assays at 10^6 PFU/mL. When incubation time was extended up to 30 minutes, all samples at 10^6 PFU/mL were positive and 1 sample at 10^5 PFU/mL was positive. Specificity was assessed using 22 unique blinded clinical samples measured in duplicate (5 Vaccinia virus samples and 6 Monkeypox virus samples). The assay correctly identified 9 of 11 orthopoxvirus clinical samples, but failed to detect one Vaccinia virus sample and one Monkeypox virus. One false-positive result was observed for 11 non-orthopoxvirus clinical samples.

Sha, J.; Endsley, J. J.; Kirtley, M. L.; Foltz, S. M.; Huante, M. B.; Erova, T. E.; Kozlova, E. V.; Popov, V. L.; Yeager, L. A.; Zudina, I. V.; Motin, V. L.; Peterson, J. W.; DeBord, K. L.; Chopra, A. K. Characterization of an F1 Deletion Mutant of *Yersinia pestis* CO92, Pathogenic Role of F1 Antigen in Bubonic and Pneumonic Plague, and Evaluation of Sensitivity and Specificity of F1 Antigen Capture-Based Dipsticks. *J. Clin. Microbiol.* **2011**, *49,* 1708-1715. DOI. 10.1128/jcm.00064-11.

Two LFAs for the detection of *Yersinia pestis* were compared in this evaluation. BioThreat Alert® and Smart™-II performed similarly and were able to detect 10^5 to 5 x 10^5 CFU/mL of the bacteria and 0.5 µg/mL for purified antigen (i.e., the surface protein from the bacteria) in PBS or infected whole mouse blood. The authors note that their limits of detection were not as low as some other studies, which could be related to the purity of the antigen or strains of *Yersinia pestis* used for the various studies.

Gessler, F.; Pagel-Wieder, S.; Avondet, M-A. Bohnel, H. Evaluation of Lateral Flow Assays for the Detection of Botulinum Neurotoxin Type A and Their Application in Laboratory Diagnosis of Botulism. *Diagn. Microbiol. Infect. Dis*. **2007**, *57*, 243–249. DOI: 10.1016/j.diagmicrobio.2006.07.017.

This study evaluated BioThreat Alert®, Smart™-II, BADD™, and RAMP® assays for their ability to detect botulinum neurotoxin in three forms: purified BoNT A, toxin complex, and toxin in the supernatant of a *Clostridium botulinum* culture. Only BADD™ and RAMP® detected purified BoNT A; LODs were 100 ng/mL for BADD™ and 50 ng/mL for RAMP®. All assays detected the BoNT A complex and SMART™-II and BioThreat Alert had greater sensitivity for the BoNT A complex (10 ng/mL) than BADD™ (100 ng/mL) and RAMP® (250 ng/mL). For the *Clostridium botulinum* culture samples, the concentration of active toxin was estimated using a mouse lethality assay. The lowest concentration of toxin in culture medium that the BioThreat Alert®, Smart™-II, and BADD™ could detect was 100 MLD/mL, while RAMP® could detect only 2500 MDL/mL. BADD™ gave a false-positive result for a culture medium that did not contain *Clostridium botulinum* or botulinum toxin.

Tomaso, H.; Thullier, P.; Seibold, E.; Guglielmo, V.; Buckendahl, A.; Rahalison, L.; Neubauer, H.; Scholz, H. C.; Splettstoesser, W. D. Comparison of Hand-Held Test Kits, Immunofluorescence Microscopy, Enzyme-Linked Immunosorbent Assay, and Flow Cytometric Analysis for Rapid Presumptive Identification of *Yersinia pestis. J. Clin. Microbiol.* **2007**, *45*, 3404–3407. DOI: 10.1128/JCM.00458-07.

This study compared an in-house immunochromatographic test (20 minute total test time), antibody-based flow cytometry (40 minute total test time), and immunofluorescence microscopy (120 minute total test time) with Tetracore's Plague BioThreat Alert® test strips (25 minute total test time), Senova's AntiBody Immuno Column for Analytical Purpose (ABICAP) columns (65 minute total test time), and Seramun's enzyme-linked immunosorbent assay (140 minute total test time). Samples were prepared

from buffer and clinical samples spiked with the fraction 1 capsular antigen of 10 different strains of *Yersinia pestis.* The BioThreat Alert® test strips positively detected a concentration of 7 x 10^3 CFU/mL in triplicate spiked buffer, but not spiked sputum (2 samples), serum (20 samples), or urine (20 samples). The immunochromatographic test positively detected all sample types at 3 x 10^3 CFU/mL. The ABICAP test and ELISA positively detected all sample types at 6 x 10^3 CFU/mL. The flow cytometry method and immunofluorescence microscopy did not produce any positive results at 5 x 10^3 and 10^3 CFU/mL, respectively. Exclusivity testing was performed using 34 clinically relevant bacteria at 5 x 10^8 CFU/mL (45 bacterial strains total). None of the assays gave false-positive results with any of the exclusivity samples.

King, D.; Luna, V.; Cannons, A.; Cattani, J.; Amuso, P. Performance Assessment of Three Commercial Assays for Direct Detection of *Bacillus anthracis* Spores. *J. Clin. Microbiol*. **2003**, *41*, 3454–3455. DOI:10.1128/JCM.41.7.3454-3455.2003.

This brief study by the Florida Department of Health Laboratory evaluated three immunoassay tests including BioThreat Alert®, Osborne Scientific's 1st generation BADD (Note: Osborne's biothreat product line was acquired by AdVnt in 2003 and the current assay is 3rd generation), and Smart™-II. The tests were evaluated for *Bacillus anthracis* (Pasteur strain) detection at quantities ranging from 10^2 to 10^6 spores (the concentration of the test samples and volume of sample applied were not reported). Only 2 to 8 samples were tested at each concentration. All test kits could detect *Bacillus anthracis* at 10^6 spores. BADD™ and Smart™-II could detect 10^5 spores; however, BioThreat Alert® detected 10^5 spores only once in eight separate assays. None of the assays could detect fewer than 10,000 spores. Tests were allowed to develop for 15 minutes, although positive results were apparent within 5 minutes. *Bacillus cereus* and *Bacillus thuringiensis* (non-threat near neighbors that could potentially result in a false-positive) were also tested twice for each test strip. No false positives were observed for the BADD™ or BioThreat Alert® tests; however, the Smart™-II tests yielded one false-positive result for *Bacillus*.

TIRF Labs: iTIRF

Phone: (919) 463-9545 *Manufacturer's website*: http://www.i-diagnostics.net/

Technology Summary

iTIRF consists of a cradle and a disposable cartridge. The technology is based on changes in fluorescence (total internal reflection fluorescence) upon target binding to the designed probes. The platform uses an iPhone or compatible smartphone to acquire and analyze the response of a microarray by employing the smartphone camera to acquire the dynamic response on the microarray.

iTIRF contains microarrays coupled with silk fibroin to increase surface area and provide better sensitivity. The microarrays can simultaneously detect proteins, nucleic acids, and metabolite biomarkers of different diseases. Minimal sample preparation is required prior to introduction of the solubilized/suspended sample into a cartridge for analysis.

Peer-Reviewed References

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

Specifications

iTIRF Immunoassay- and Nucleic Acid-Based Probe Total Internal Reflectance Fluorescence Biodetector

Biothreat Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD**
Ricin toxin	*Ricinus communis*	Not reported
Cholera toxin	*Vibrio cholerae*	Not reported
Botulism (botulinum toxin)	*Clostridium botulinum*	Not reported
Chemical agents	Various	Not reported
RNA/DNA markers	Various	Not reported

Assay time: *~ 5 minutes.*
Required sample preparation: *Minimal.*
Automatic results display: *Yes.*
Unit weight: *1 lb.*
Power: *Rechargeable batteries.*
Cost: *Assays – $2-$40 each; application development kit – $9900.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *24 months from date of manufacture.*

TIRF Labs: TIRF Sense

Phone: (919) 463-9545 *Manufacturer's website*: http://www.tirf-labs.com/

Technology Summary

TIRF Sense is a portable multi-purpose chem-bio sensor designed for use in the field and mobile labs. TIRF Sense is marketed in the areas of food safety, military, biodefense, and agriculture. A nanoengineered bioassay, based on total internal reflectance fluorescence, is used in a microfluidic system.

The instrument uses a microarray-based platform that can detect nucleic acids, proteins, and metabolite markers of diseases, as well as toxins and chemical agents. The sensor detects molecular markers using DNA molecular beacons and aptamer- or antibody-based beacons. Samples must be in liquid form to be introduced into the instrument. The device is remotely reprogrammable and can perform standard or user-defined analyses. The sensor is equipped with Wi-Fi, Bluetooth, USB, cellular connectivity, and a GPS system. TIRF microarrays include internal positive and negative controls, in addition to luminescence standards for normalization and calibration.

Peer-Reviewed References

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

Specifications

Immunoassay- and Nucleic Acid-Based Probe Total Internal Reflectance Fluorescence Biodetector

TIRF Sense		
Disease/Toxin	**Causative Agent/Source**	**LOD**
Ricin toxin	*Ricinus communis*	Not reported
Cholera toxin	*Vibrio cholerae*	Not reported
Botulism (botulinum toxin)	*Clostridium botulinum*	Not reported
Chemical agents	Various	Not reported
RNA/DNA markers	Various	Not reported

Assay time: *5-10 minutes.*
Required sample preparation: *Minimal.*
Automatic results display: *Yes.*
Unit weight: *5.5 lb.*
Power: *Rechargeable batteries.*
Cost: *Assay – $20-$400 each; instrument – $35,000.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *1-12 months from date of manufacture.*

5.0 PCR-Based Detection Systems

PCR-based assays detect specific organisms based on their DNA sequence. During PCR, short pieces of DNA from the biothreat organism are amplified, creating millions of DNA copies from just a few hundred starting molecules. PCR assays are designed to recognize regions of DNA that are unique to the biothreat organism(s).

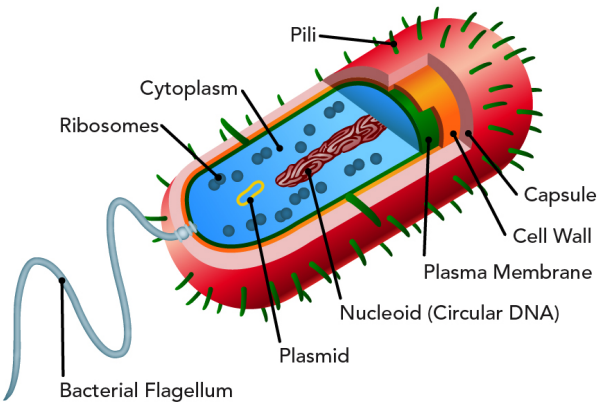
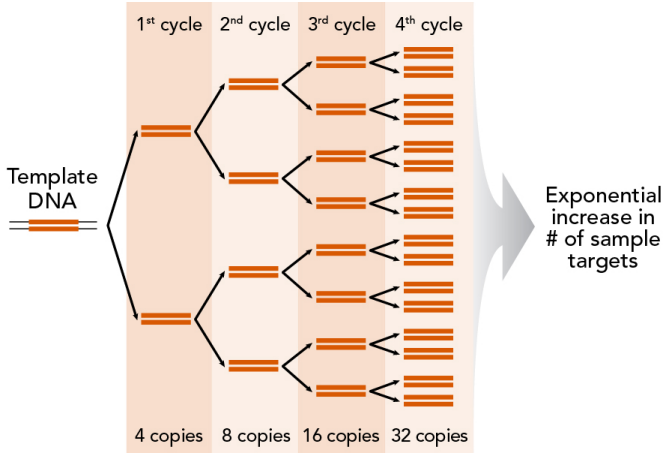


Illustration of a bacterial cell. All cells and viruses contain DNA or RNA that can be used to detect and identify them.

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 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 (sometimes included) to swab a suspicious powder and solubilize or suspend the white powder in a compatible buffer. Depending on the system, various degrees of sample preparation or cartridge manipulation may be required including pipetting, manual mixing, or centrifugation. In the instrument, the sample/reagent mixture is cycled between high and low temperatures to amplify the biothreat agent DNA. Many PCR assay formats result in a final dye-labeled product DNA, which is measured by integrated optical components (usually fluorescence-based). Most assays contain an internal positive control to ensure the system components and reagent cartridges are performing as specified. Failure of an internal positive control can indicate problems with the system hardware/software, cartridge reagent issues, or presence of interfering substances in the sample.

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). Very few field-based PCR systems have integrated sample preparation to concentrate DNA and remove PCR inhibitors; however, because PCR is very sensitive, a sample can often be significantly diluted after sampling to reduce the effects of potential inhibitors on the reaction. Few published studies are available that assess the impact of environmental samples and hoax powders on PCR-based assays when little or no sample preparation is conducted. The most significant disadvantages of PCR-based approaches are relatively long assay times (typically 30 to 60 minutes) and, for some systems, relatively high costs. Some assays detect more than one "target" (a specific region on a biothreat agent) and



PCR can amplify a single piece of DNA ("target") to make millions of copies in 30 to 60 minutes.

positive detection of multiple targets in a sample can improve confidence that the biothreat agent is actually present. PCR assays will not detect toxins, unless the toxin preparation contains DNA from the source organism (e.g., castor bean DNA in a crude preparation of ricin toxin).

Hand-Portable PCR-based Systems

- Assay cost: $8 to $19 (1-agent test) to $185 to $200 (10- to 16-agent multiplex test)
- Instrument cost: $899 to $39,500
- Examples:
  - FilmArray® (BioFire Diagnostics)
  - RAZOR® EX (BioFire Diagnostics)
  - one3™ (Biomeme)
  - POCKIT™ (Gene Reach USA)
  - POCKIT™ Micro (Gene Reach USA)
  - T-COR 8™ (Tetracore)

BioFire Defense, LLC: FilmArray®

Phone: (801) 262-3592 *Manufacturer's website*: http://www.biofiredefense.com

Technology Summary

The FilmArray® is a fully automated, multiplexed PCR-based platform. The system consists of four components: the loading station, reagent pouch, instrument/detector, and laptop computer.

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 by mechanical agitation with ceramic beads within 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 µL 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 biothreat agents by monitoring the fluorescence quenching of a double-stranded DNA binding probe. The melting curve is dependent upon 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® PCR Instrument and Associated Components

Biothreat 16-Agent Assay		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*	500 spores/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	1 µg/mL
Brucellosis	*Brucella* species	1000 CFU/mL
Glanders/ Melioidosis	*Burkholderia mallei/pseudomallei*	1000 CFU/mL
Hemorrhagic fever	Ebola virus	10,000 PFU/mL
Hemorrhagic fever	Marburg virus	10,000 PFU/mL
Plague	*Yersinia pestis*	500-5000 GE/mL**
Q fever	*Coxiella burnetii*	1000 CFU/mL
Ricin toxin	*Ricinus communis*	1 µg/mL
Smallpox	Variola virus (species)	10,000 PFU/mL
Smallpox	Variola virus (genus)	10,000 PFU/mL
Tularemia	*Francisella tularensis*	5000 GE/mL**
Typhus	*Rickettsia prowazekii*	1000 CFU/mL
Viral encephalitis	Venezuelan equine encephalitis virus	10,000 PFU/mL
Viral encephalitis	Eastern equine encephalitis virus	10,000 PFU/mL
Viral encephalitis	Western equine encephalitis virus	10,000 PFU/mL

*Reported by manufacturer and peer-reviewed publications.
** GE=genome equivalent (GE/mL ~ CFU/mL)

Assay time: *60 minutes.*
Required sample preparation? *Yes, minimal.*
Automatic results display? *Yes.*
Unit weight: *20 lb.*
Power: *110V AC.*
Cost: *Assay – $1110/6 pack ($185 each, $12/agent, $7/target); instrument – $39,500.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *6 months from date of manufacture.*

BioFire Defense has a biothreat panel that simultaneously tests a single sample for the presence of 27 targets (16 agents). Initial testing of the FilmArray® biothreat pouch by the Pacific Northwest National Laboratory (PNNL) (publication listed below) for detection of *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis* genomic DNA samples supports the manufacturer's sensitivity claims. In addition, an

FDA-approved respiratory, sepsis, and gastrointestinal panel is commercially available. While numerous peer-reviewed publications exist for the other panels, they are not listed below.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system) and ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices).

Peer-Reviewed References

Seiner, D. R.; Colburn, H. A.; Baird, C.; Bartholomew, R. A.; Straub, T.; Victry, K.; Hutchison, J. R.; Valentine, N.; Bruckner-Lea, C. J. Evaluation of the FilmArray® System for Detection of *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*. *J. Appl. Microbiol.* **2013**, *114*, 992-1000. DOI: 10.1111/jam.12107.

This study evaluated the sensitivity and specificity of the BioFire Diagnostics FilmArray® system using the biothreat pouch for the detection of three strains each of *Bacillus anthracis*, *Francisella tularensis,* and *Yersinia pestis* DNA and a brief evaluation of ability of the FilmArray® system to detect *Bacillus anthracis* spores (Sterne strain). The FilmArray® software algorithm requires the positive detection of all included targets for *Bacillus anthracis* and one target for *Francisella tularensis* or *Yersinia pestis* before a positive identification of the biothreat agent is indicated. The biothreat pouch includes three targets for *Bacillus anthracis* (pX01, pX02, and a chromosomal target) and two targets each for *Francisella tularensis* (FTT2 and FTT3 targets) and *Yersinia pestis* (YpT1 and YpT3 targets). Results indicate that the sensitivity for these assays is approximately 5000 genome equivalents (GE) per mL or lower. At concentrations of 5000 to 500,000 GE/mL, positive identification occurred in 53 of 54 samples tested with 1 false-negative occurring for *Francisella tularensis* strain holarctica 425 at a concentration of 5000 GE/mL. For concentrations of 250 to 500 GE/mL, 23 of 24 samples were correctly identified as *Bacillus* species, but *Bacillus anthracis* was not indicated because not all targets were detected. For *Francisella tularensis* and *Yersinia pestis,* positive results were obtained in 40 of 48 samples. To assess the potential for false-positive results, DNA from three near-neighbor organisms was tested (three strains for *Bacillus anthracis and Francisella tularensis* and four strains for *Yersinia pestis*) at concentrations ranging from 5×10^4 to 5×10^6 GE/mL. No false-positive results were obtained for any near neighbors in the 60 exclusivity samples that were tested. However, near-neighbor strains having a particular biothreat target (e.g., pX01) did produce a positive result for that target (but did not generate a positive detection result for a biothreat agent because the other signatures were not present). While no false-positive biothreat agents were observed (which requires the positive detection of all targets for that particular biothreat), four false-positive detections occurred for a *Bacillus anthracis* target when testing *Yersinia* species-containing samples (either inclusivity or near-neighbor/exclusivity samples). One *Francisella tularensis* sample (500,000 GE/mL) generated a false-positive result, indicating the presence of *Staphylococcus aureus* (i.e., the presence of two biothreats was indicated rather than just the actual *Francisella tularensis* present). Of the 38 blank samples analyzed, only one false-positive detection resulted, which was likely due to a pouch failure. Of 226 assays performed, two software crashes of unknown cause resulted in failures of those assays, requiring re-analysis of the samples. Inspection of the raw data indicated that the false-positive was due to the incorrect software interpretation of a melt curve, as no amplification was observed in the saved data. Six replicate samples containing 500 spores/mL of *Bacillus anthracis* (Sterne strain), which only contains the pX01 and chromosomal targets, but not the pX02 target, were also analyzed. One of the two targets was detected in all samples and both targets were detected in three samples.

BioFire Defense, LLC: RAZOR® EX

Phone: (801) 262-3592 *Manufacturer's website*: http://www.biofiredefense.com

Technology Summary

The RAZOR® EX is a PCR-based system for pathogen detection in the field. The RAZOR® EX system is packaged in an over-the-shoulder carrying case and the complete system and case weighs 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 used by a wide range of first responders (e.g., police and fire departments).

The system performs PCR on a crude sample solubilized from a swab. Typically, a single sample is injected into the "reagent pouch," which contains all of the assay reagents (e.g., primers, probes, enzymes, and buffers) needed for the PCR reaction. Sample dilution is used to reduce the effects of possible PCR inhibitors and the system relies on thermal disruption to release DNA from cells and viruses. No additional clean-up of the sample is performed. In preparing the pouch for a run, several required manual preparation steps 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), and 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. Other assays include a food screen 3-agent assay (*Campylobacter*, *Listeria monocytogenes* and *Salmonella*), a water screen 3-agent assay (*Cryptosporidium*, *E. coli* O157:H7 and *Salmonella*), and a 3-target biothreat assay for anthrax.

Specifications

RAZOR® EX PCR Instrument, Reagent Pouch, Buffer, and Syringes

Biothreat Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
10-Agent Biothreat Assay		
Anthrax	*Bacillus anthracis*	1000 CFU/mL
Brucellosis	*Brucella* species	30,000 CFU/mL
Bacterial infection	*E. coli* O157:H7	3000 CFU/mL
Salmonellosis	*Salmonella*	350 CFU/mL
Botulism (botulinum toxin)	*Clostridium botulinum*	3000 CFU/mL
Plague	*Yersinia pestis*	100 CFU/mL
Q fever	*Coxiella burnetii*	1000 CFU/mL
Ricin toxin	*Ricinus communis*	1 µg/mL
Smallpox	Variola virus	238 PFU/mL
Tularemia	*Francisella tularensis*	100 CFU/mL
1-Agent (3 Target) Biothreat Assay		
Anthrax	*Bacillus anthracis* (pX01 target 1)	1000 CFU/mL
Anthrax	*Bacillus anthracis* (pX01 target 2)	1000 CFU/mL
Anthrax	*Bacillus anthracis* (pX02 target)	1000 CFU/mL
*Reported by manufacturer and peer-reviewed publication.		

Assay time: *30 minutes.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes.*
Unit weight: *11 lb.*
Power: *Battery (8 runs/charge).*
Cost: *Assay – $200/10-agent assay ($20/agent), $180 for 1-agent/3 target assay ($60/target); instrument – $38,500.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *6 months from date of manufacture.*

Biothreat assays have been evaluated by end-users in various countries (testing was sponsored by manufacturer and reported on their website). The BioThreat 10-target screen kit tests for *Bacillus anthracis*, *Brucella*, *Coxiella*, *E. coli*, *Francisella tularensis, Salmonella, Yersinia pestis*, smallpox, ricin toxin, and botulinum toxin. In addition, the RAZOR® platform has been independently evaluated in a DHS-sponsored Stakeholder Panel on Agent Detection Assays (SPADA) (see AOAC reference 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 the 2479 samples tested provided the expected results (99% success with 95% confidence). The anthrax assay evaluated in these studies is no longer commercially available, so the applicability of these results to the currently offered assays is unknown.

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system) and ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices).

Peer-Reviewed References

No peer-reviewed publications were found that evaluate the use of currently offered assays for the detection of biothreat agents. Publications listed below evaluated a 3-target assay for anthrax (different than the one listed in the specification table) that is no longer available.

Hadfield, T.; Ryan, V.; Spaulding, U. K.; Clemens, K. M.; Ota, I. M.; Brunelle, S. L. Razor Ex Anthrax Air Detection System for Detection of Bacillus Anthracis Spores from Aerosol Collection Samples: Collaborative Study. *J. AOAC Int.* **2013,** *96*, 392-398. DOI: 10.5740/jaoacint.CS2012-06.

This report summarizes the results of a collaborative study that tested the RAZOR EX at 3 sites by 12 operators for the ability to measure *Bacillus anthracis* in simulated aerosol collection buffer. The RAZOR assay pouch used in this study is no longer commercially available. The pouch detected three targets of *Bacillus anthracis* (pX01, pX02, and a chromosomal target). Samples were prepared using 1 mg/mL of standardized dust in PBS and spiked with either 2000 spores/mL of *Bacillus anthracis* Ames strain (expected positive result) or 20,000 spores/mL of *Bacillus cereus,* a near-neighbor expected to yield a negative result. Because this study was evaluating the RAZOR EX as a tool for the laboratory analysis of aerosol filters, significant sample preparation was done prior to detection. Note that this complex level of sample preparation would likely not be performed by first responders analyzing suspicious powders in the field (typically only powder dilution or automated sample preparation is performed in the field). Prior to analysis on the RAZOR EX, all samples were processed as follows: 1) a sample was pipetted into a tube containing disruptor beads and placed in a vibrating Disruptor Genie® for 5 minutes to lyse the spores; 2) the sample was pipetted into a tube containing magnetic beads and pipette-mixed to bind the DNA in the sample onto the magnetic beads; 3) a magnetic bead capturing tool was used to capture the magnetic bead-DNA complex after mixing for 30 to 45 seconds; 4) the complex was transferred to a sample well containing wash solution and the process was repeated twice, each time transferring the bead-DNA complex to a new sample well; 5) the bead-DNA complex was transferred to a well containing elution buffer and allowed to incubate at room temperature for at least 2 minutes to dissociate the DNA from the magnetic beads; 6) the magnetic beads were captured from the elution buffer using the magnet tool and discarded, leaving just DNA in the elution buffer; 7) the elution buffer sample was transferred to the RAZOR sample buffer bottle. A total of 144 samples were analyzed. Each operator analyzed 12 *Bacillus anthracis* (Ames strain) samples (144 samples total) and 12 *Bacillus cereus* samples (143 samples total). All 144 *Bacillus anthracis* (Ames strain) samples were positive for all three targets, and all 143 *Bacillus cereus* (near-neighbor) samples produced negative results, as expected. This performance met the requirements of AOAC Standard Method Performance Requirement 2010.003, developed by SPADA.

Spaulding, U. K.; Christensen, C. J.; Crisp, R. J.; Vaughn, M. B.; Trauscht, R. C.; Gardner, J. R.; Thatcher, S. A.; Clemens, K. M.; Teng, D. H. F.; Bird, A.; Ota, I. M. RAZOR® EX Anthrax Air Detection System. *J. AOAC Int.* **2012***, 95*, 860-891. DOI: 10.5740/jaoacint.11-521.

This reference summarizes the AOAC Method Developer (MD) and independent laboratory validation (ILV) studies for the RAZOR® EX Anthrax Air Detection System. The RAZOR® EX pouch that was used in this study is no longer commercially available. The pouch detected three targets of *Bacillus anthracis* (pX01, pX02, and a chromosomal target). Because this study was evaluating the RAZOR® EX as a tool for laboratory analysis of aerosol filters, significant sample preparation was done prior to detection. Note that this complex level of sample preparation would likely not be performed by first responders analyzing suspicious powders in the field (typically only powder dilution is performed in the field). Prior to analysis on the RAZOR® EX, all samples were processed as follows: 1) a sample was pipetted into a tube containing disruptor beads and placed in a vibrating Disruptor Genie® for 5 minutes to lyse the spores; 2) the sample was then pipetted into a tube containing magnetic beads and pipette-mixed to bind the DNA in the sample onto the magnetic beads; 3) a magnet tool was used to capture the magnetic bead-DNA complex by mixing for 30 to 45 seconds; 4) the complex was transferred to a sample well containing wash solution and the process was repeated twice, each time transferring the bead-DNA complex to a new sample well; 5) the bead-DNA complex was transferred to a well containing elution buffer and allowed to incubate at room temperature for at least 2 minutes to dissociate the DNA from the magnetic beads; 6) the magnetic beads were captured from the elution buffer using the magnet tool and discarded, leaving just DNA in the elution buffer; 7) the elution buffer sample was transferred to the RAZOR® sample buffer bottle. The MD studies included inclusivity/exclusivity testing using 15 strains of *Bacillus anthracis* and 20 strains of closely related (near-neighbor) DNA at 2000 GE/mL (equivalent to approximately 2000 spores per mL) and 20,000 GE/mL respectively. All inclusivity and exclusivity strains gave expected results. Liquid and air collection filters were used as matrices with and without added standardized dust and spiked with 2000 spores/mL of *Bacillus anthracis* (Ames strain) spores (inclusivity) or 20,000 spores/mL *Bacillus cereus* (near-neighbor) for matrix studies. A total of 96 replicates of each sample type were analyzed (768 samples total). The pX01 target test gave expected results for all replicates of each organism in all matrices. The pX02 target test yielded expected results for all but one replicate of *Bacillus cereus* strain E33L in a dust/filter matrix. The chromosomal target test gave expected results for all but two samples and matrices. Two clean filter samples containing *Bacillus anthracis* produced false-negative results, thus failing the SPADA acceptance criteria of no more than one unexpected result in 96 replicates. Environmental interference was assessed using five soil types (0.1 g/mL with filter present; 7 mg/mL for PBS) and 23 powders/chemicals (0.1 mg/mL with filter present; 7 µg/mL for PBS) with 2000 spores/mL of *Bacillus anthracis* and 20,000 spores/mL of *Bacillus cereus*. No false-positive or false-negative results were observed for any samples. Upper and lower limits of detection were determined using DNA to be 10 ng/test volume and 50 fg/test volume, respectively. Robustness was evaluated by deliberately varying the storage time of lysed samples (0 and 2 hours), storage time of purified samples prior to loading into pouch (0 and 4 hours), storage time of pouches prior to loading samples (0 and 30 minutes), and storage time of loaded pouches prior to PCR analysis (0 and 30 minutes). None of these variations resulted in a failure to detect the presence of *Bacillus anthracis* for any sample. Product consistency and reliability were evaluated by testing different manufacturing lots, and over the time period of the consumable products' (DNA extraction kit and pouch) shelf-lives. No significant impact was found for these variables. Instrument variability was examined by testing six different RAZOR® EX instruments and found to be acceptable. The following ILV studies were also

conducted. Inclusivity/exclusivity and matrix study testing was done as in the MD studies with no unexpected results; however, the pX01 target was only detected in 93 of 96 replicates in the clean filter matrix, thus failing the SPADA acceptance criteria of no more than one unexpected result in 96 replicates. However, it is important to note that the instrument did meet SPADA acceptance criteria for all of the dirty filter samples, as well as the inclusivity and exclusivity sample testing. One explanation for the poorer performance on clean filters is that DNA recovery from clean filters may be lower than desired due to adherence of biological material on the clean filters. In addition, clean filters likely represent an unrealistic field situation. An ILV environmental interference study was also conducted in the same fashion as the MD study. All samples yielded expected results except that one subsoil interfered with the detection of pX01. ILV LOD studies achieved reliable detection from 1 pg/test volume to 10 ng/test volume. Overall, the RAZOR® EX Anthrax Air Detection System was recommended for *Performance Tested Method* certification and submission for *Official Method of Analysis* collaborative study.

Biomeme: one3™

Phone: (267) 519-9066 *Manufacturer's website*: www.biomeme.com

Technology Summary

The Biomeme one3™ device is a portable PCR-based platform for performing real-time PCR (RT-PCR) and isothermal molecular analysis. The platform consists of four components: a thermocycler operated by an iPhone, field-test kits, iOS mobile software, and a cloud-based data management system for data back-up, analysis, and real-time dissemination of results.

Samples are suspended in a small volume of nuclease-free water, transferred to an assay tube containing lyophilized reaction components, mixed and then transferred into a 3-well strip along with two controls. The operator places the 3-well strip into the one3™ system, closes the lid, and begins the test via the iOS application on the iPhone. The average test time is between 40 and 120 minutes. After the analysis is complete, the results are displayed on the iPhone as a cycle threshold (Ct) value, which is the number of cycles required for the measured fluorescence signal to reach a level that indicates a target biothreat is present. Future software upgrades will automate the determination of biothreat absence/presence based on programmed Ct threshold values. Detailed results, including amplification plots and raw data, can be exported from the iPhone. All test data are GPS-tagged and encrypted, and can be securely synced via Wi-Fi or cellular connection to a web portal for real-time remote monitoring of test results and further analysis. The Biomeme system is configurable with other real-time PCR, RT-PCR, and isothermal assays, which may be run on the platform.

Available biothreat assays are listed in the specifications table. In addition to Biomeme's kits, which include several food-safety threat agents, SRC Acumen has developed a series of biothreat field test kits specifically for use on the Biomeme platform. SRC Acumen assays are designed to

Specifications

Biomeme one3™

Biothreat 1-Agent Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD***
Anthrax	*Bacillus anthracis*; 1 chromosomal, 1 plasmid	200-1000 spores/mL
Ricin toxin and Abrin toxin	*Ricinus communis* and *Abrus precatorius*	1-600 ng/mL
Botulinum toxin A	*Clostridium botulinum*	1-600 ng/mL
SEB	*Staphylococcus aureus*	1-600 ng/mL
Plague	*Yersina pestis*	20 CFU/mL
Tularemia	*Francisella tularensis*	200-1000 CFU/mL
Brucellosis	*Brucella* species	200-1000 CFU/mL
Q fever	*Coxiella burnetii*	200-1000 CFU/mL
Viral Encephalitis	Venezuelan equine encephalitis virus	1500-7200 PFU/mL
Pan Orthopox (including smallpox)	Variola virus	1500-7200 PFU/mL
Ebola	Ebola virus	Not reported
Epsilon toxin	*Clostridium perfringens*	Not reported
Melioidosis	*Burkholderia mallei*	Not reported
Psittacosis	*Chlamydophila psittaci*	Not reported
Typhus fever	*Rickettsia* (incl. R. *prowazekii*)	Not reported
Cholera	*Vibrio cholerae*	Not reported
Cryptosporidiosis	*Cryptosporidium* (incl. *C. parvum)*	Not reported
*Reported by manufacturer		

Assay time: *~ 1 hour.*
Required sample preparation? *Minimal.*
Automatic results display? *No (user interprets displayed value to determine if a biothreat is present).*
Unit weight: *1 lb (including iPhone and battery).*
Power: *Battery and AC wall adapter.*
Cost: *Assay – $760 field kit/ ($19/test); instrument – $4950.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *Up to five years from date of manufacture (at room temperature).*

work in the presence of a heavy interferent load, such as soil and salts, so that environmental sample cleanup is not required.

The one3™ weighs 1.0 lb (including iPhone and battery) and supports up to six tests on a single battery charge. It also comes with a 110V-240V AC wall adapter. The device has three reaction wells and supports FAM and SYBR (commonly used fluorescent DNA binding dyes) and loop-mediated isothermal amplification protocols. There are currently no internal controls, so the recommended procedure for each run (of three tubes) is to use one tube for the sample, one as a positive control, and one blank.

Peer-Reviewed References

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

GeneReach USA: POCKIT™ Nucleic Acid Analyzer

Phone: (617) 749-8500 *Manufacturer's website*: http://www.genereach-us.com

Technology Summary

The POCKIT™ is a PCR-based platform that can measure up to eight samples in parallel (including separate controls). Pipetting and centrifugation steps are required before analysis.

The POCKIT™ uses convection PCR (i.e., the PCR tube is heated to have a temperature gradient and the solution moves by convection in the gradient, resulting in sample thermal cycling). Results are displayed as a simple positive/negative on an LCD screen. Raw fluorescence data for the background and sample are also stored on a SD memory card and can be viewed to aid interpretation of suspect sample results.

Two detection wavelengths are used: 520 or 550 nm (or both). The instrument requires 100-240 V, 50/60 Hz AC power and could be adapted to operate with lithium batteries or a car charger.

Available biothreat assays are listed in the table. In addition, users can develop their own tests by following the POCKIT™ primer/probe design rules and using available sample tubes and supplied buffer. The assays do not include internal positive controls, so the user must run any controls as additional samples. Therefore, three reaction tubes would be required to run one positive control and one blank in parallel with each sample.

Specifications

POCKIT™ PCR Instrument, Centrifuge, and Pipette

Available Biothreat Assays		
Disease/ Toxin	**Causative Agent/Source**	**Detection Limit***
Anthrax	*Bacillus anthracis* (pXO1 target)	2600 spores/mL
Anthrax	*Bacillus anthracis* (pXO2 target)	8000 spores/mL
Anthrax	*Bacillus anthracis* (chromosomal target)	4000 spores/mL
Salmonellosis	*Salmonella* species	11,200 CFU/mL
Brucellosis	*Brucella* species	1000 CFU/mL
Brucellosis	*Brucella melitensis*	10,000 CFU/mL
Brucellosis	*Brucella abortus*	10,000 CFU/mL
Ricin toxin	*Ricinus communis*	2800 CFU/mL
Viral Infection	Dengue virus	1200 CFU/mL
Viral Infection	Middle east respiratory syndrome coronavirus (ORF1a)	3400 CFU/mL
Viral Infection	Middle east respiratory syndrome coronavirus (upE)	6000 CFU/mL
Viral Infection	Rift valley fever virus, L segment	2000 CFU/mL
Viral Infection	Rift valley fever virus, M segment	6400 CFU/mL
*Reported by manufacturer		

Assay time: *60 minutes.*
Required sample preparation? *Moderate.*
Automatic results display? *Yes, as positive or negative.*
Unit weight: *4.6 lb.*
Power: *110V AC.*
Cost: *Assay – $380/48 reactions ($8 each); instrument – $8,000.*
Additional costs: *Sample collection supplies.*
Assay shelf-life*: 24 months from date of manufacture.*

The management system governing the manufacture of this product is ISO 9001:2008-certified (specifies the requirements of a quality management system) and ISO 13485:2003-certified (specifies the requirements of a quality management system for medical devices).

Peer-Reviewed References

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

GeneReach USA: POCKIT™ Micro Nucleic Acid Analyzer

Phone: (617) 749-8500 *Manufacturer's website*: http://www.genereach-us.com

Technology Summary

The POCKIT™ Micro Nucleic Acid Analyzer is a small, portable PCR-based platform that can measure four samples in parallel. After several liquid transfer steps, the sample vials are placed in the instrument and analysis is completed in under 30 minutes for DNA targets.

The POCKIT™ Micro uses convection PCR (i.e., the PCR tube is heated to have a temperature gradient and the solution moves by convection in the gradient). The process does not require thermal cycling optimization/programming. Results are displayed as a simple positive/negative on an LCD screen.

After sample collection (sample collection supplies not provided), the sample is solubilized and the solution is mixed with PCR primer/probe reagents and transferred into a reaction tube. The reaction tube is briefly swung to remove bubbles and placed into the instrument for measurement.

The instrument can analyze between one and four samples in parallel, and it can operate with battery for at least five assays when fully charged. The instrument utilizes a single channel detection wavelength of 520 nm. The assays do not include internal positive controls, so the user must run any controls as additional samples. Therefore, three reaction tubes would be required to run one positive control and one blank in parallel with each sample.

Specifications

POCKIT™ Micro PCR Instrument

Available Biothreat Assays		
Disease/ Toxin	**Causative Agent/Source**	**Detection Limit***
Anthrax	*Bacillus anthracis* (pXO1 target)	2600 spores/mL
Anthrax	*Bacillus anthracis* (pXO2 target)	8000 spores/mL
Anthrax	*Bacillus anthracis* (chromosomal target)	4000 spores/mL
Salmonellosis	*Salmonella* species	11,200 CFU/mL
Brucellosis	*Brucella* species	1000 CFU/mL
Brucellosis	*Brucella melitensis*	10,000 CFU/mL
Brucellosis	*Brucella abortus*	10,000 CFU/mL
Ricin toxin	*Ricinus communis*	2800 CFU/mL
*Reported by manufacturer.		

Assay time: *~30 minutes.*
Required sample preparation? *Moderate.*
Automatic results display? *Yes, as positive or negative.*
Unit weight: *0.84 lb.*
Power: *Rechargeable Battery (at least 5 runs when fully charged).*
Cost: *Assay – $380/48 reactions ($8 each; detects one biothreat target per assay); instrument – $900 (single channel for DNA targets, instrument for RNA targets is under development).*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *24 months from date of manufacture.*

Peer-Reviewed References

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

Tetracore, Inc.: T-COR 8™

Phone: (240) 268-5400 *Manufacturer's website*: http://www.tetracore.com

Technology Summary

The Tetracore T-COR 8™ is a hand-portable, battery-powered, PCR instrument. The system is capable of running eight independent samples simultaneously. At present, the instrument uses four (expandable to six) detection wavelengths. Each cartridge contains up to three biothreat targets and an internal positive control. Expansion of the instrument to six detection channels will provide the potential for two additional biothreat agents to be added to each cartridge. 2-agent and 3-agent cartridges are currently available.

The instrument uses a touch screen interface and has an integrated barcode scanner. PCR amplification curves can be viewed in real time and data is stored on a secure data storage card. The system battery life is approximately 4 hours.

The sample is added to a buffer solution, and the resulting mixture is added to the cartridge containing all the reagents needed for the DNA amplification reaction. Samples can be amplified using either isothermal amplification chemistries or real-time PCR, and each sample well amplification temperature cycling program can be independently programmed. The results are viewable directly on screen and the instrument is "cloud" ready and can transmit data and be remotely accessed via a wireless or ethernet network. The T-COR 8 was preceded by the T-COR 4, a 4-channel PCR system with 1-agent assays, which included an internal control. The T-COR 4 has been discontinued.

Specifications

T-COR 8™ PCR Instrument

Biothreat Assays		
Disease/Toxin	**Causative Agent/Source**	**LOD**
2-Agent Biothreat Assays		
Abrin	*Abrus precatorius*	Not reported
Ricin	*Ricinus communis*	Not reported
Anthrax	*Bacillus anthracis* (pX01 target 1)	Not reported
Anthrax	*Bacillus anthracis* (pX01 target 2)	Not reported
Smallpox	Variola virus	Not reported
3-Agent Biothreat Assays		
Botulism (botulinum toxin)	*Clostridium botulinum* (Toxin A & B)	Not reported
Melioidosis	*Burkholderia*	Not reported
SEB	*Staphylococcus aureus*	Not reported
Brucellosis	*Brucella* species	Not reported
Plague	*Yersinia pestis*	Not reported
Tularemia	*Francisella tularensis*	Not reported

Assay time: *20-45 minutes, depending on isothermal versus real-time PCR temperature programming.*
Required sample preparation? *Minimal.*
Automatic results display? *Yes.*
Unit weight: *10 lb.*
Power: *Battery (4 hours).*
Cost: *Assay – $768/64 pack of 2- or 3-agent assays ($12 each; $4-$6/target or agent); instrument – $28,500.*
Additional costs: *Sample collection supplies.*
Assay shelf-life: *12 months from date of manufacture.*

Peer-Reviewed References

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