Battelle Memorial Institute

B.M.D. S.p.A.

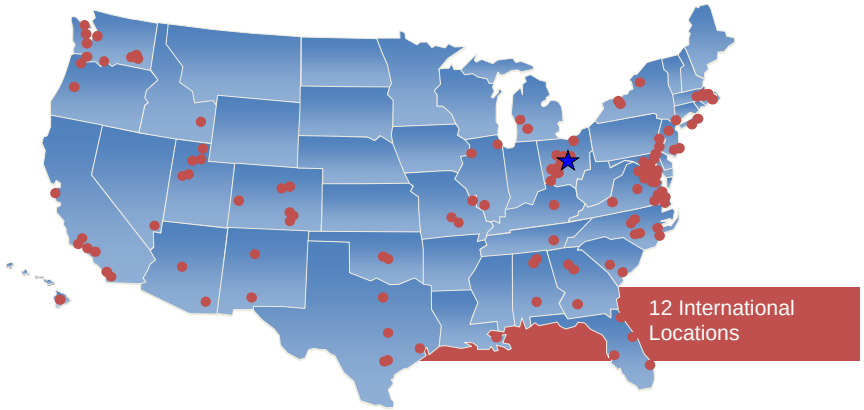
TradeWays USA Ltd

Spectroscopic Species Identification of Biological Warfare Agents

Introducing the Resource Effective Bioidentification System - REBS

Battelle Memorial Institute

- Non-profit charitable trust – began operations in 1929
- 77 years of research and development leadership
- Conducts $6.5 billion in annual R&D
- 20,000 employees worldwide (including labs we co-manage)
- Over 5,000 projects for 1,100 industrial & government customers
- Develops between 50 and 100 patented inventions annually
- More than 150 locations
- &D business in more than ries



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REBS Briefing Outline

- Why REBS?
- System Description
- REBS Process Overview
- Testing Overview and Results
- Modes of Operation
- Benefits

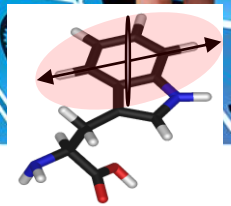
REBS is a Cost Saving Alternative to Traditional Biological Identification Systems

Why REBS?

- **Low Life Cycle Cost**
- Near Real-time Identification
- No Consumable Liquids
- Autonomous Operation
- **Aerosol Collection Directly to Identification**
- IDs all BWAs and aerosol CWAs
- **Ability to Add New Threats in 24 hours**
- Low False Alarm Rates
- Non-Destructive Analysis

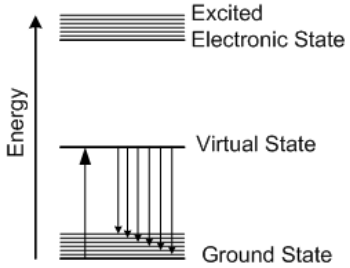
- Portable, 12 hour battery operation
- Archive Sample Suitable for, and network ready
- PCR Confirmation

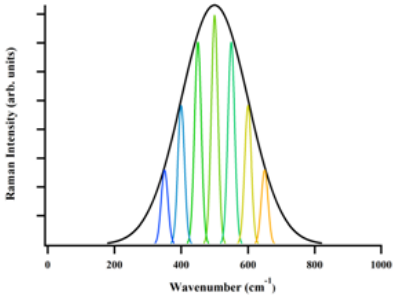
Raman Spectroscopy



Application of Raman in REBS

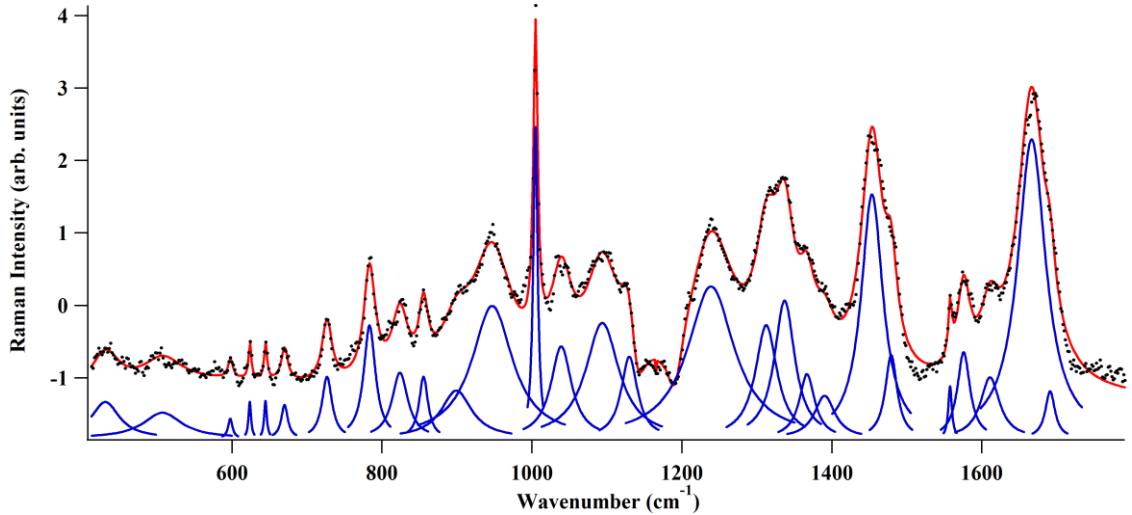
- Light/matter interaction results in a Raman shifted photon specific to the **molecular bonds**
- **Molecule structural conformation** influences spectra and are used for biological identification
- DNA/RNA, spore coat, capsule, cell membrane, intracellular contents all contribute to the measured spectra
- **Bonds and conformation** both contribute to identification
- Spectral measurement does not degrade viability/activity
- Cell viability, in some instances, is discernible in spectra
- **Single cell/particle** measurements removes interferant confusion
- Factors influencing growth (extrinsic variability) are discarded
- **No primers, probes, liquids reagents or substrates needed**





Accurate, agile and economical point identification system

Biological Spectral Signatures Comprised from Chemical Moieties

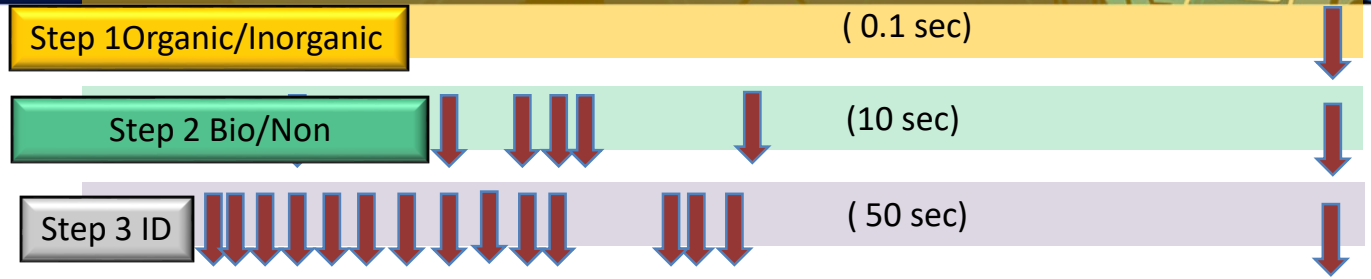


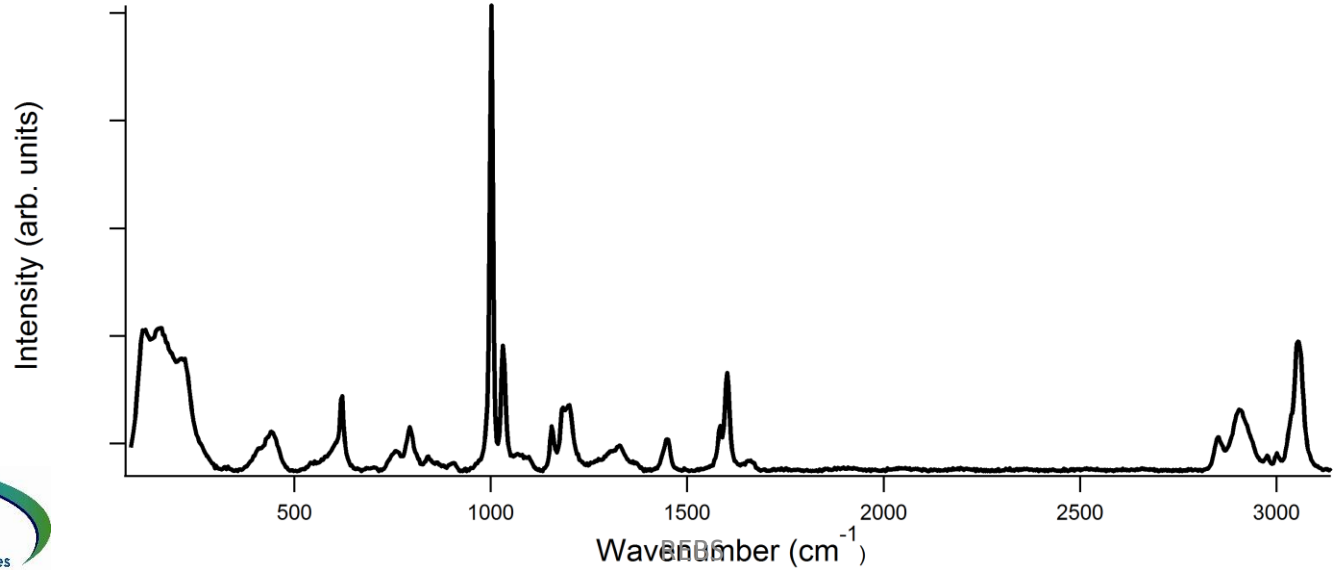
- Vegetative *Bacillus subtilis* spectral signature shows many Raman shifted peaks that can be attributed to different types of molecular bond resonances
- Each molecular bond resonance experiences a local environment due to the biological conformational structure. Conformation influences the natural resonance peak position, peak width and peak amplitudes.

REBS Process Animation

REBS Digital Threat Identification Process

Triaged approach to Identification





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REBS Testing Overview

Location (Sponsor)	Date	Type	Result
El Paso, Texas	6/2009 to 7/2009	Background sampling	**Zero False Positives**
Dugway, Utah	10/2009 to 11/2009	TRE Simulant challenge in realistic environment	**ID sensitivity - 25 ACPLA**
Battelle Columbus, Ohio	11/2009 to 01/2010	Identifier TRE Samples, ID performance	**100% Identification**
Battelle Columbus, Ohio	6/2009	CWA (particle) ID feasibility	**Dual threat feasibility proven**
Adelphia, Maryland	9/2010	TRE shoot off with simulants	**Selected for Competitive Prototyping**
Dugway, Utah	2/2011 to 7/2011	ASEC and ABT testing with irradiated materials, unknown biological, near-neighbors and interferants	**Sensitivity, Specificity, and False Alarm Performance**
Boston, Massachusetts	10/2012 to 6/2014	Simulant release in actual environment	**Zero False Positives, 100% identification of simulant release**
Aberdeen, Maryland	6/2013 to 8/2013	Blinded trials, live agent in background, simulant in battlefield interference	**100% live agent identification, Zero False Positives**
Dugway, Utah	9/2014 to present	Open air chemical, biological and explosives in desert environment	**Dual biological and chemical simulant detection**

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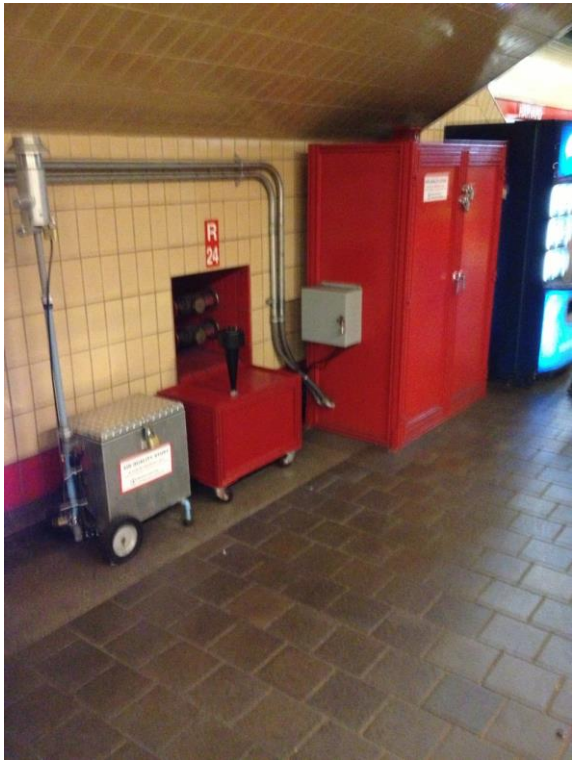
Live Agent Sensitivity Results

- The average time to alarm was 22 minutes.
- No aerosol challenge was misidentified as any other threat in the spectral library.
- Simulated ambient background (bacteria, mold, and Arizona test dust) did not interfere or inhibit the sensitivity limits of identification.
- Additional ambient breeze tunnel backgrounds trials did not interfere or inhibit the sensitivity limits of identification.

REBS Boston Subway Operational Test and Evaluation

- REBS systems configured with threat libraries that contained: BWA agents and BWA simulants.
- Systems configured for 30-minute duty-cycle. Collection and analysis occur in parallel, allowing continuous monitoring of the environment.
- All systems rated for -10-50C temperature range
- Battelle's approach was to maintain systems to support the scheduled releases. The systems were set to run for a two-week interval after the release.
- System support and consumable replenishment after the two-week interval was conducted as resources allowed.



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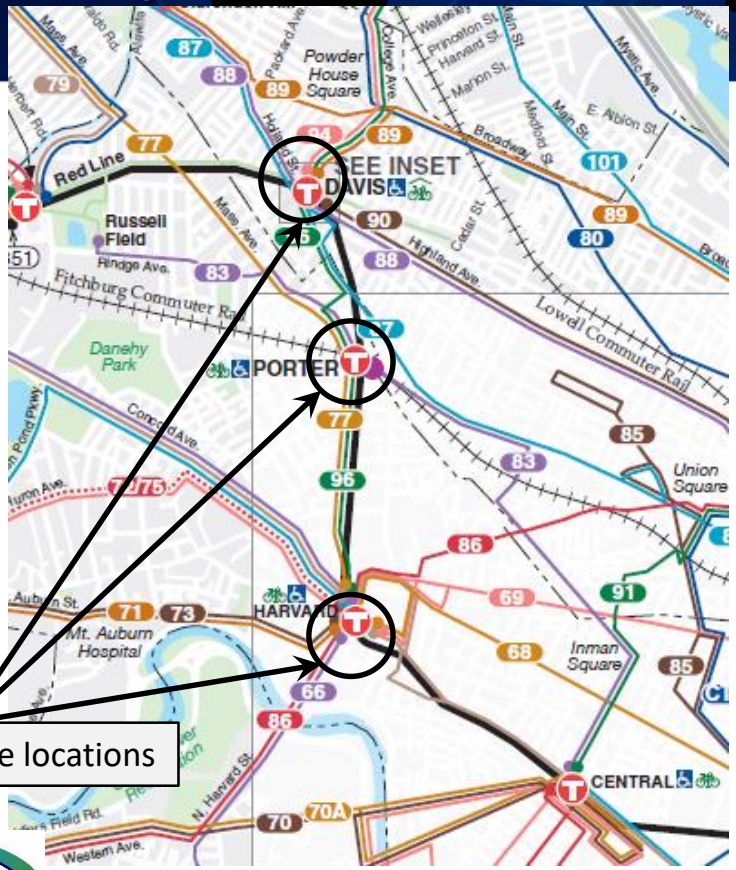
Boston Subway Biodetection Test



- Three REBS systems installed into the MBTA system at encircled locations indicated on the map.
- Trains moving between stations after releases
- Biological simulant disseminated on the station platform.
- Measurement of simulant concentration at the three locations versus time.
- Systems enclosed in secondary container for security
- Davis to Porter 0.7 miles (walking)
- Porter to Harvard 1.1 miles (walking)
- Davis to Harvard 1.8 miles (walking)

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Actual Environmental Testing Performance Summary

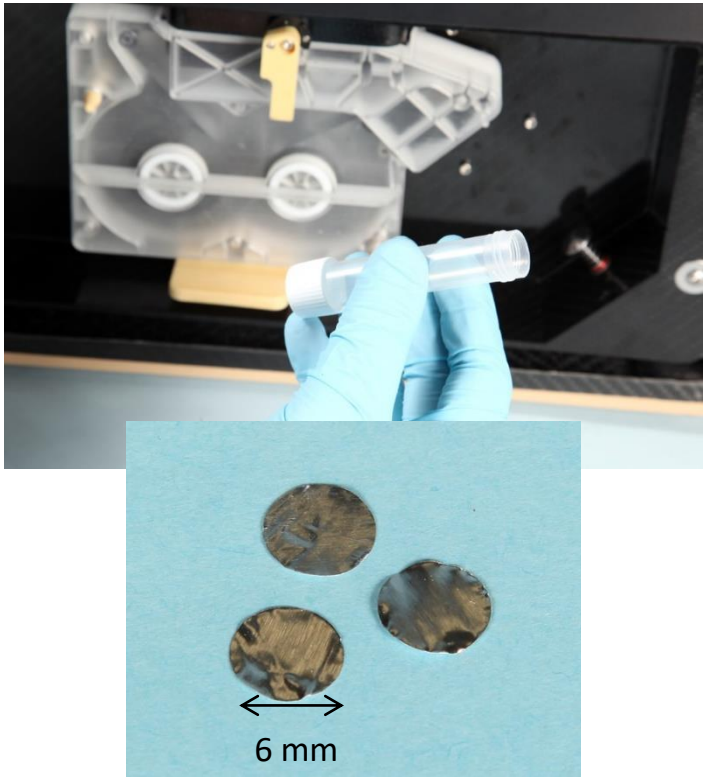
Location	El Paso, TX	Boston, MA Subway
Duration	06/2009 – 07/2009	10/2012 – 4/2013
Hours	740	2,454
Samples	651	5,860
Particles	1,494,557	3,498,363
CH+	5499	15,488
Bio+	63	493
Simulant +	N/A	56
Threat+	0	0

- Limit of Identification, zero false positives
- Threat Database: Alarmed for BWA simulants only. Zero false alarms

Demonstrated Zero False Positives over Extended Deployments

Orthogonal Sample Conformation

- Upon a positive threat identification, REBS automatically archives the sample
- Archived sample chad is ejected into a 6 ml vial. Vial is serialized with the cassette and logged into the system operational database.
- The ‘Chad’ can be eluted with near 100% extraction efficiency
- The extracted material is then either cultured or amplified via PCR
- Applied Biosystems TaqMan assay has been successfully applied
- Samples of the extracted volume placed into loaded microwell plates
- Approximately 30 CT cycles result in confirmation from 1ml extracted volume



Resource Effective BioIdentification System

Benefits

- *Reliable and rapid bio-identification*
- *Ability to readily add "new" agent threats*
- *Significantly reduced operational and consumables costs and effort – minimized life cycle costs and logistics tail*
- *Rugged design for multiple user environments*
- *Minimal False Alarms*

Specific Features

- Inexpensive cost of consumables per system
- Hundreds of threats and threat mixtures identifiable simultaneously
- Continuous/autonomous operation with near real-time identification for a month
- Battery operable for 12 hour missions
- Network ready for arrayed sensor operation and remote monitoring
- Adaptable to changing backgrounds
- Sample archival compatible with genetic (PCR) "gold standard" identification methods

SARS-CoV-2 Detection

Battelle deployed REBS units for monitoring in hospital environments.

- Developed library for hospital studies that contains SARS-CoV-2, several other human coronaviruses, and background particles
  - Generated spectral signatures for multiple common coronaviruses
  - Incorporated signature of SARS-CoV-2 virus
  - Demonstrated ability to differentiate between SARS-CoV-2 and other coronaviruses
- Placed REBS into various hospital areas to detect possible virus shedding by patients
  - Placed REBS into common areas and COVID+ patient rooms
  - Measured rate of SARS-CoV-2 and background particle detection
  - Negative detections in some rooms in agreement with other analysis methods
- Additional studies planned to fully develop and validate SARS-CoV-2 detection capability.

Any questions?