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Meeting User Needs: Developing Field-Deployable Biodetection Systems Using a Micro-Separations Approach

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Sandia National Laboratories

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Biodetection Technologies 2011 Conference

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Developing fieldable instruments has challenges in addition to meeting laboratory requirements

Laboratory requirements:

- **High sensitivity**
- **High specificity**
- **User-friendly or automated**
- **Robust**

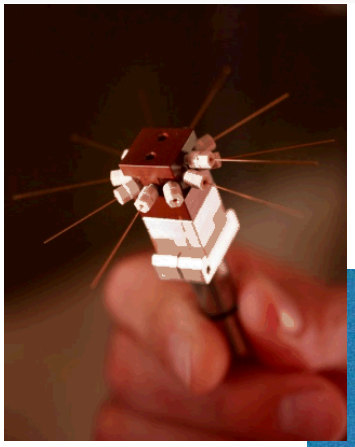
Field-portable requirements:

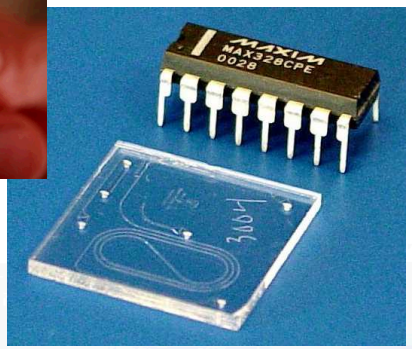
- **High sensitivity**
- **High specificity**
- **User-friendly or automated**
- **Robust**
- **Portable**
- **Low-power**
- **Stable reagents**
- **Low cost**

Simply shrinking lab-based equipment for field use is not always feasible, and may lead to a loss in performance and high costs

Sandia "Born Small" approach: Portable systems using miniaturized assays & components

- **Sandia began developing systems for microfluidics-based analysis in the late 1990s**
  - At that time, the "lab-on-a-chip" concept was just emerging
  - Componentry needed to interface to microfluidic chips and create portable systems **did not exist**
- **Out of this effort grew a capability for developing field-portable systems for biodetection and medical diagnostics**
  - Programmatic focus arose from our national security mission
- **Major program sponsors: Internal Sandia LDRD (start), DOE, DHS, DoD, NIH**





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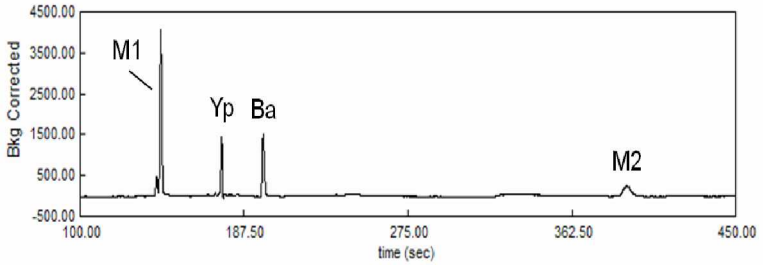
Our approach exploits microseparations for detecting biological threat agents

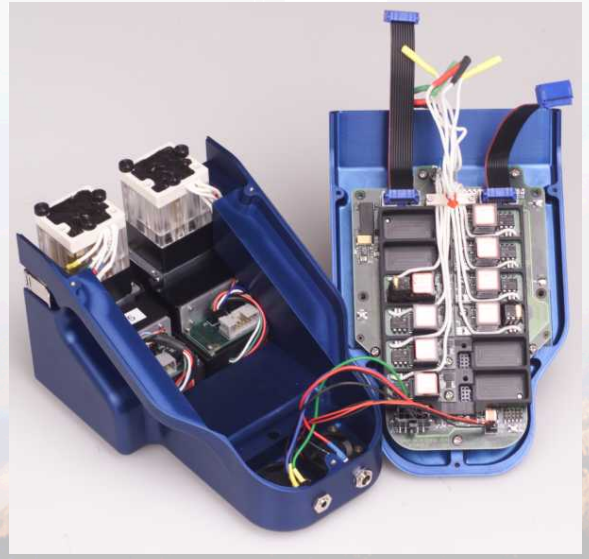


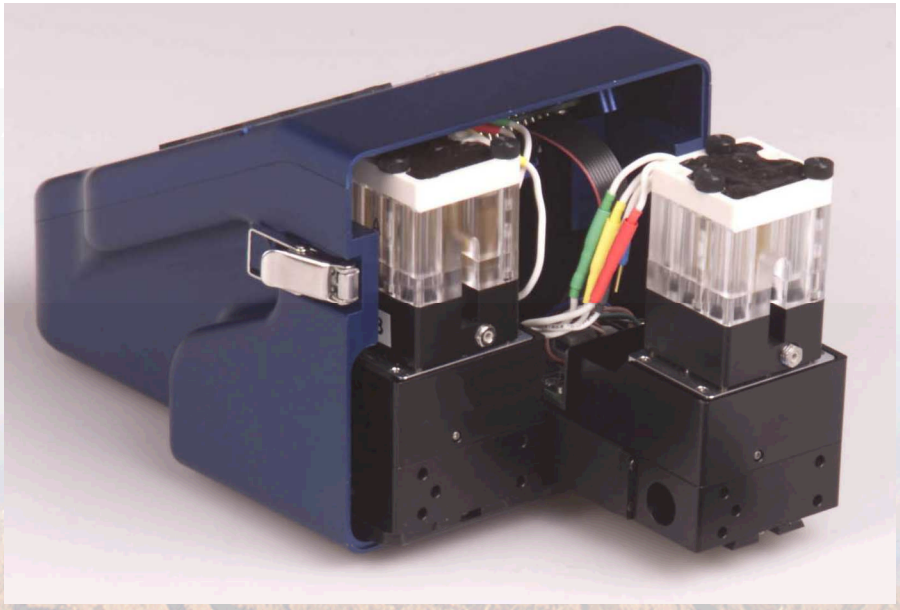
To meet field-portable requirements:

- **For high sensitivity, utilize LIF and preconcentration techniques**
- **For high specificity, utilize orthogonal analyses and develop microseparations-based nucleic acid and antibody assays**
- **Develop automated sample preparation appropriate for a wide variety of threats**
- **Incorporate engineering controls to provide reliable system performance**
- **Utilize components designed to be low power**
- **Utilize lower cost components (lasers, plastic chips, reagents)**
- **Buffers and dyes are stable and cheap; very small volumes needed**

Integrated Biodetection Platforms –µChemLab™

- Handheld instrument for first responders
- Modular packaging
- Two analysis modules
- Integrated control and data analysis





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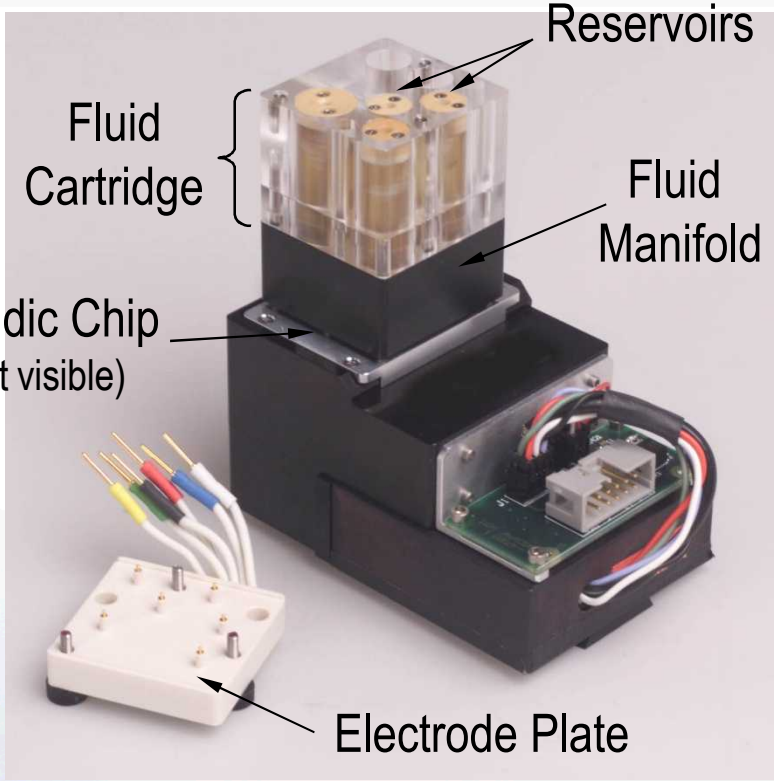
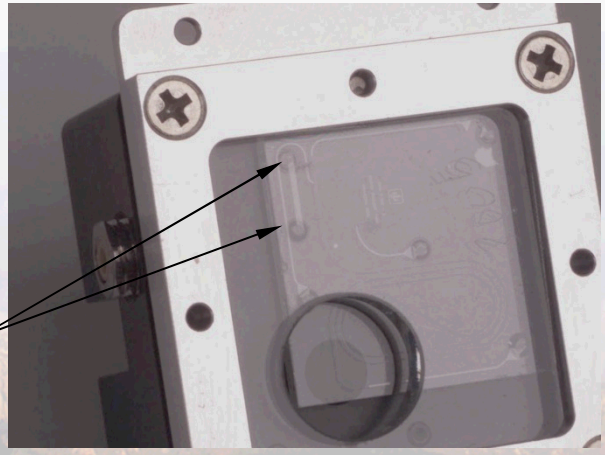
Polymeric fluid manifold provides reproducible leak-tight interface to the microfluidic chip



Separation Module

- **O-ring face seal enables simple chip installation**
- **Reservoirs housed in a fluid cartridge hold running solutions**
- **Capillaries in fluid manifold provide fluid connection between reservoir and chip**
- **Electrode plate connects high voltage source to solutions**



Renzi *et al, Anal. Chem.* **2005,** *77*, 435-441

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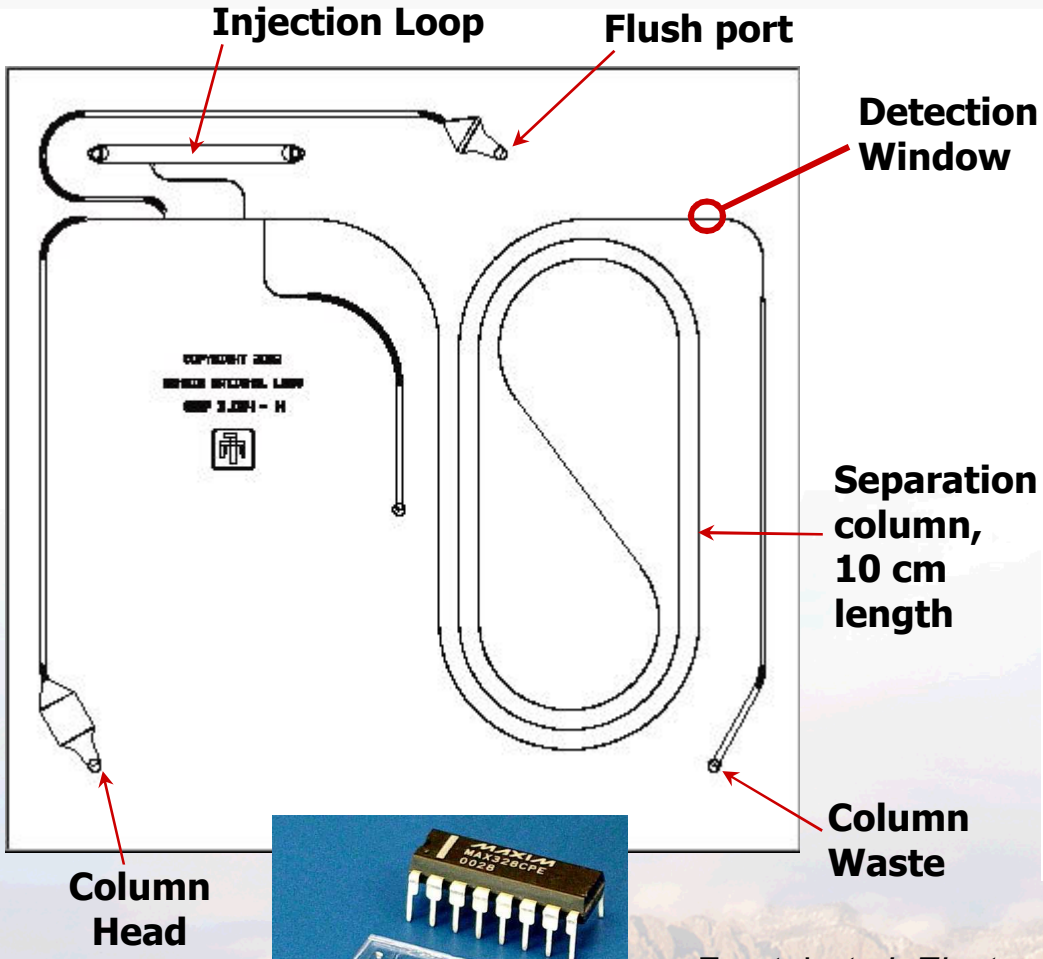
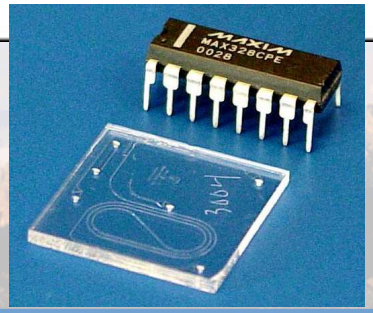
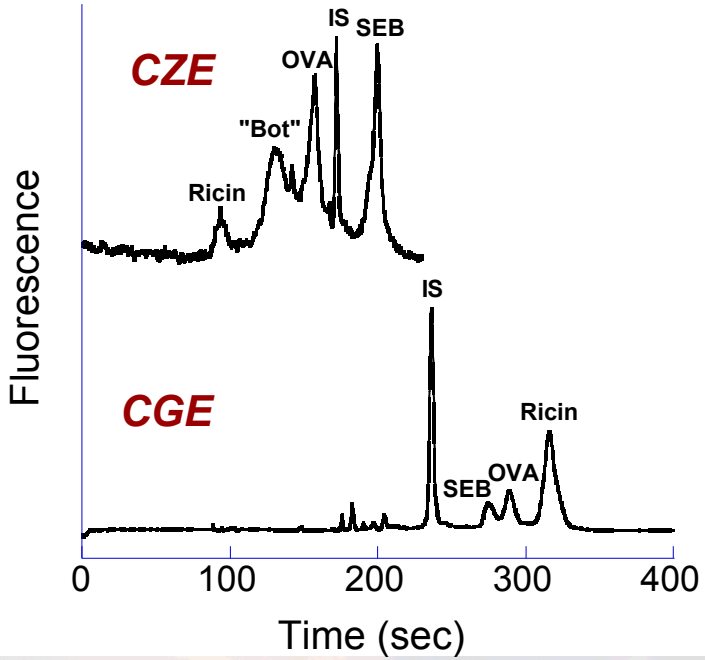
Dual CE/LIF platforms enable rapid parallel chip-based analyses at nano-molar sensitivities





Biotoxin detection uses two separation methods for improved detection reliability



Fruetel *et al, Electrophoresis* **2005**, *26*, 1144–1154
Renzi *et al, Anal. Chem.* **2005,** *77*, 435-441

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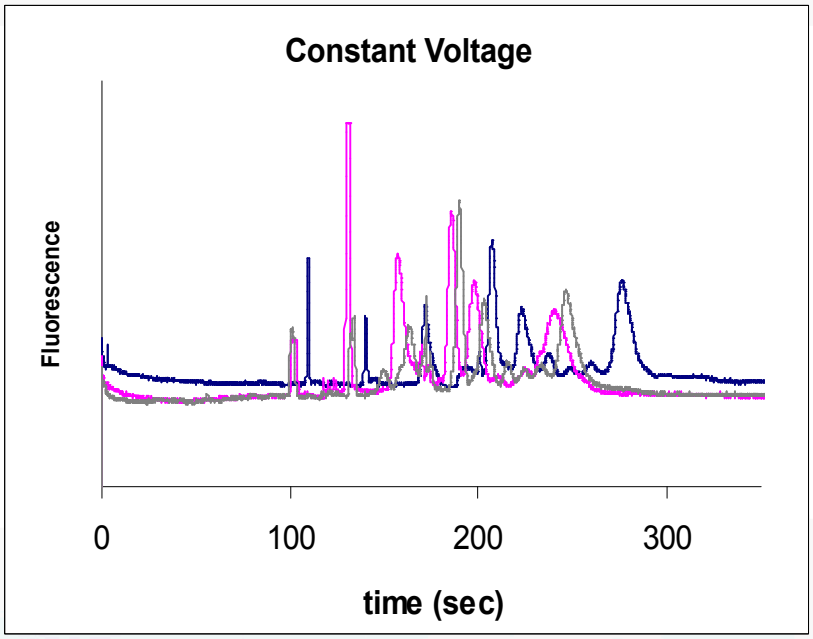
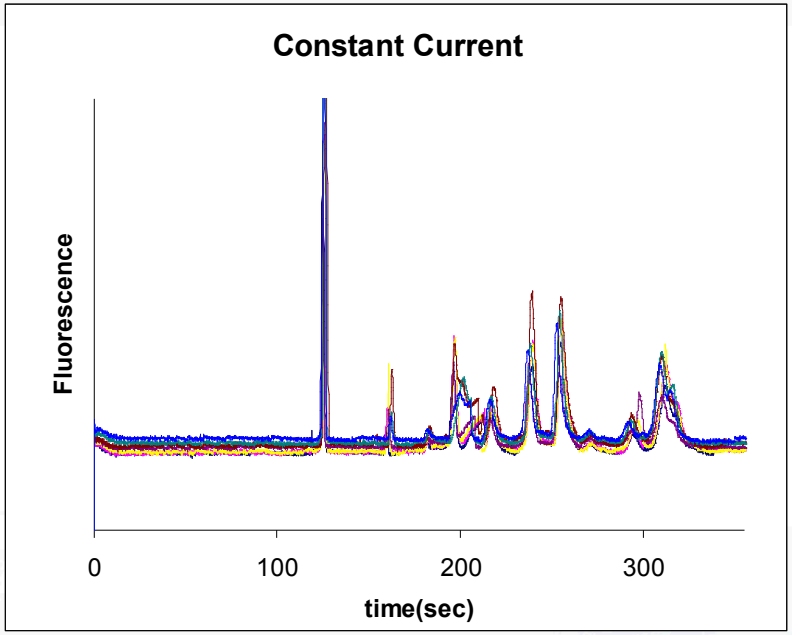
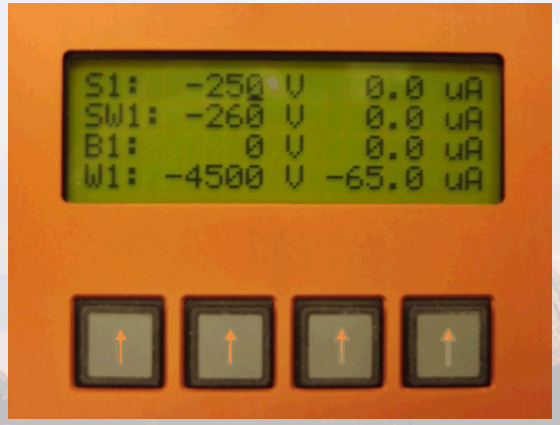
Current monitoring and control improves run-to-run reproducibility and provides system health



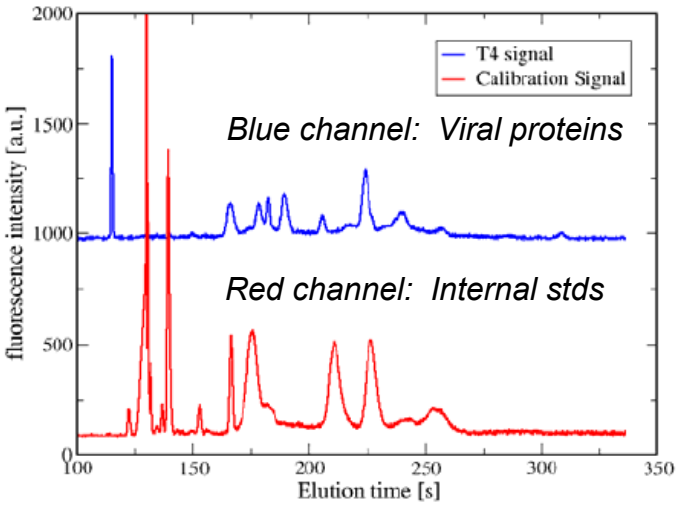


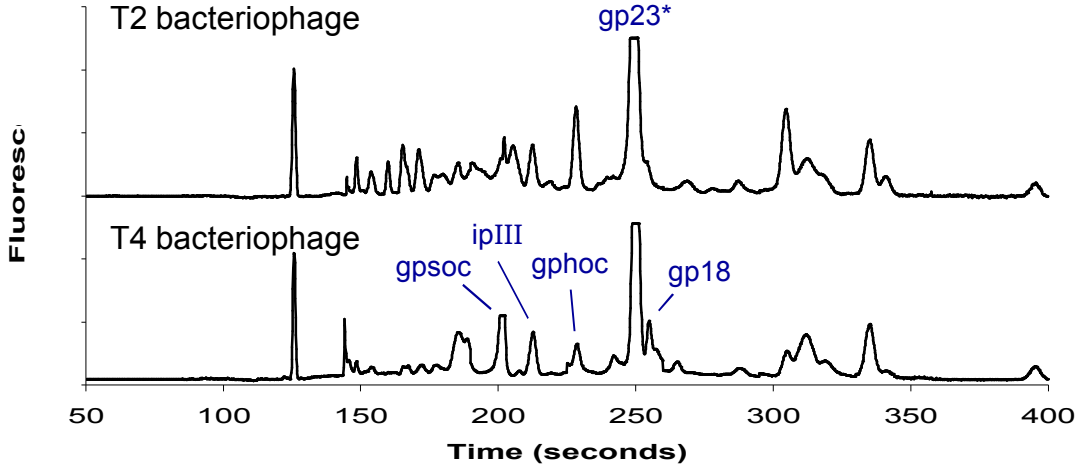


run #	cck	lact	CA	OVA	BSA	IgG
1	162.2	199.5	216.7	236	251.7	308.5
2	161.7	199.5	216	236.5	252.6	308.5
3	162.3	201.5	216.6	238.1	254	309.5
4	162.5	196.5	218	238.8	254.5	310
5	160.5	196	216	237.5	253.7	309.5
6	161.2	196.5	217.5	238.8	254.7	311
7	160.5	196.5	217.8	239.2	255	311
8	160	196.6	217.8	239.3	255	312
9	159.5	196	217	239	254.5	311.5
Average	161.1556	197.45	217.04	238.07	254.07	310.1667
S.D.	1.086406	1.967655	0.727553	1.160508	1.119573	1.274755
RSD	**0.674**	**0.997**	**0.335**	**0.487**	**0.441**	**0.411**

Identification of viruses using microfluidic protein profiling and Bayesian classification

Two-color fluorescence detection enables molecular weight assignments through use of internal standards





Performance of the Bayesian classifier on non-training samples in terms of the number of runs where the Bayes factor >5 (considered decisive evidence of the agent being present)

virus	total number of runs	no. of runs used as training data	classification results: no. of runs, not used for training: Bayes factor > 5	Bayes factor <0 ("other")
T2	11	6	4	1
T4	18	9	9	0
vaccinia	29	12	16	1
MS2	41	17	24	0
EBV	13	8	4	1
RSV	14	5	9	0
total	126	57	66	3

Fruetel *et al, Anal. Chem.* **2008,** 80, 9005–9012

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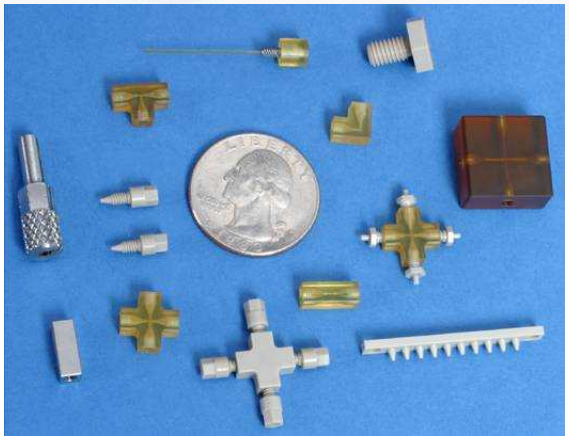
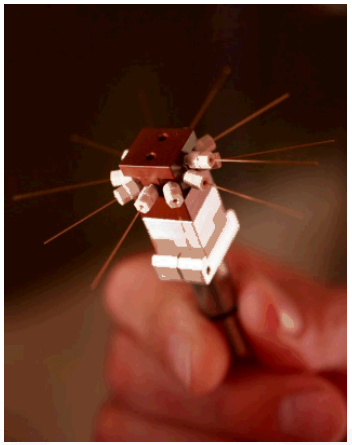
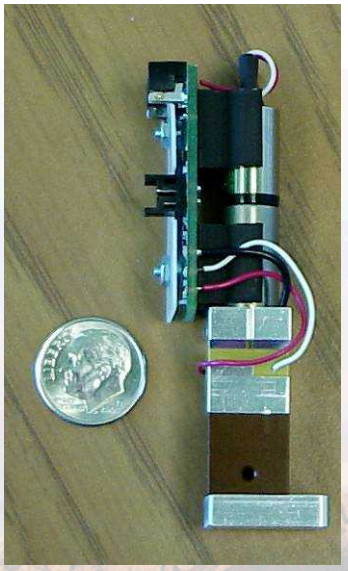
Enabling Technologies - Microfluidics



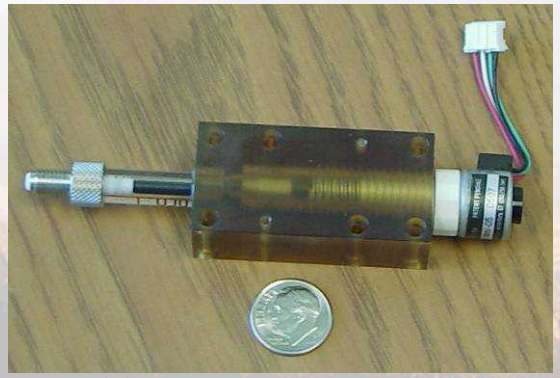
CapTite™ Fluidic Fittings
- One piece design
- Reusable
- Finger or tool tightened
- Up to 10 KSI



10-port valve



Miniature valves
- 10 KSI capability
- Electrically actuated
- Low swept volumes (~10 nL)



Syringe pumps
- Linear stepper motor
- Controlled fluid metering
- Bidirectional

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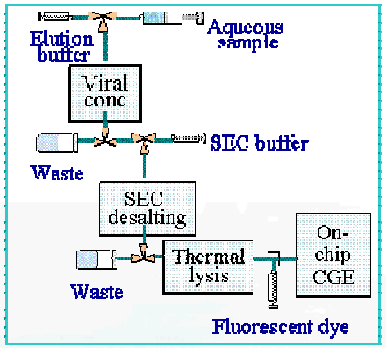
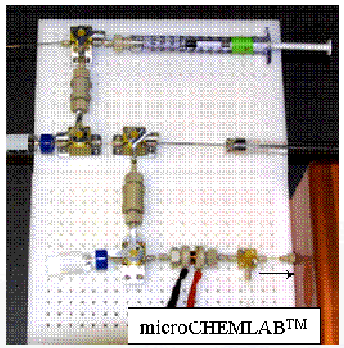
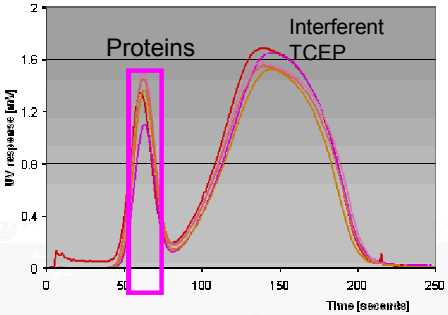
Enabling Technology: Modular microscale sample processing for ease of use and robustness

- **Need: Bridge the gap between mL-sized samples and nL-sized chip-based analysis in a format that enables portability and unattended operation**
- **Developed cartridges for processing µL sample volumes in seconds to minutes**
  - Concentration
  - Fractionation
  - Filtering
  - Sieving
  - Desalting
  - Contaminant removal
  - Digestion
  - Buffer exchange
  - Cell lysis
- **Has been applied to proteins and intact organisms**

Packed-bed cartridges

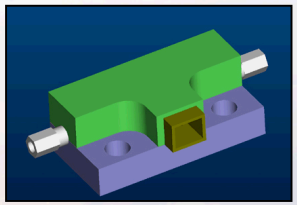


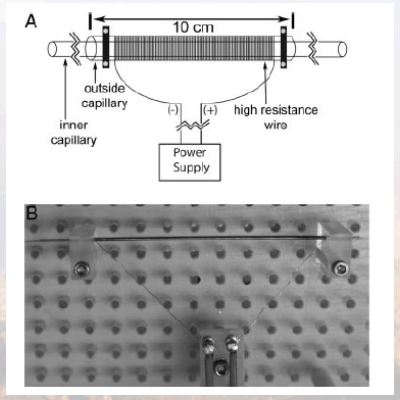




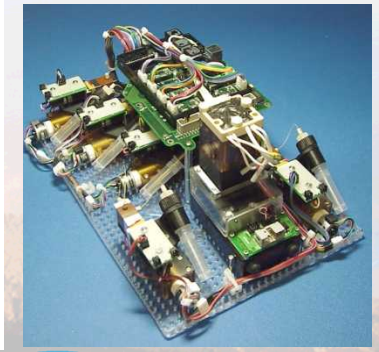
On-line SEC

Thermo/chemical lysis





Integrated systems



Chirica *et al, Anal. Chem.* **2006**, 78, 5362-5368
Hukari *et al, Electrophoresis* **2010**, 31, 2804–2812

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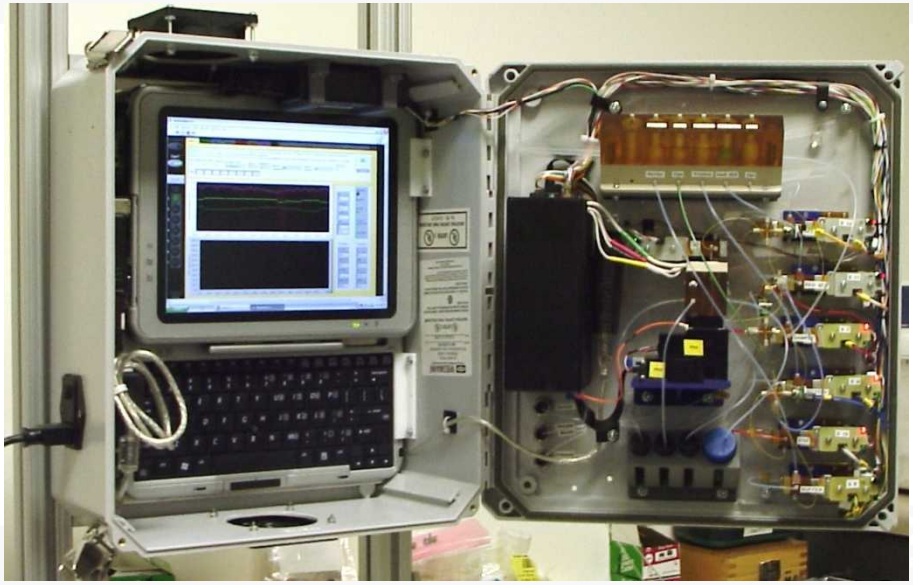
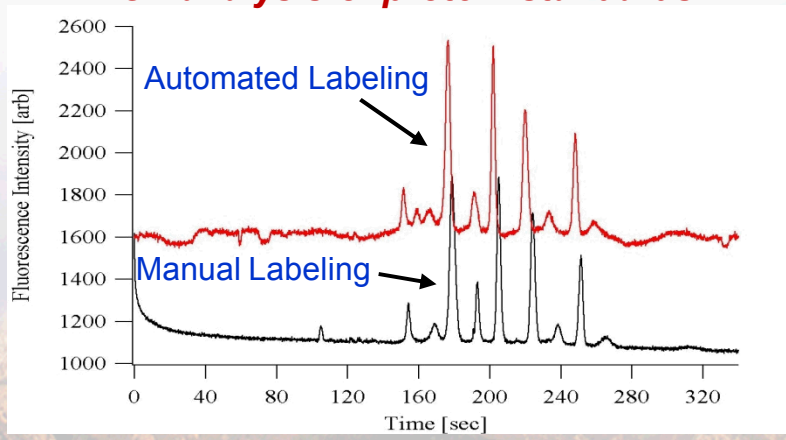
Integrated Biodetection Platforms – Unattended Water Sensor



VanderNoot *et al*, *Electrophoresis* **2010**, 31, 2632–2640

- **Continuous water monitoring for utilities**
  - 30 day unattended operation
  - Analysis every 30 minutes
  - Detects biotoxins
- **Funded through CRADA with Tenix and CH2MHill**
- **Automated: sampling of water main, fluorescent labeling of proteins, and data analysis**

CE analysis of protein standards



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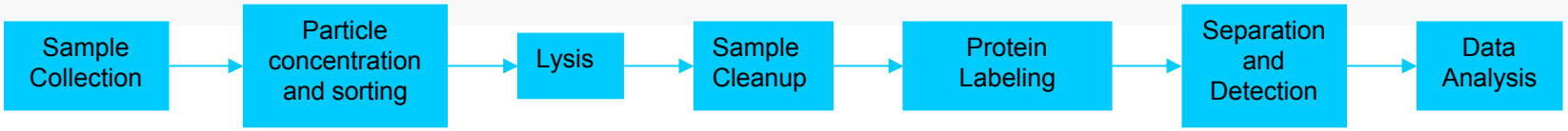
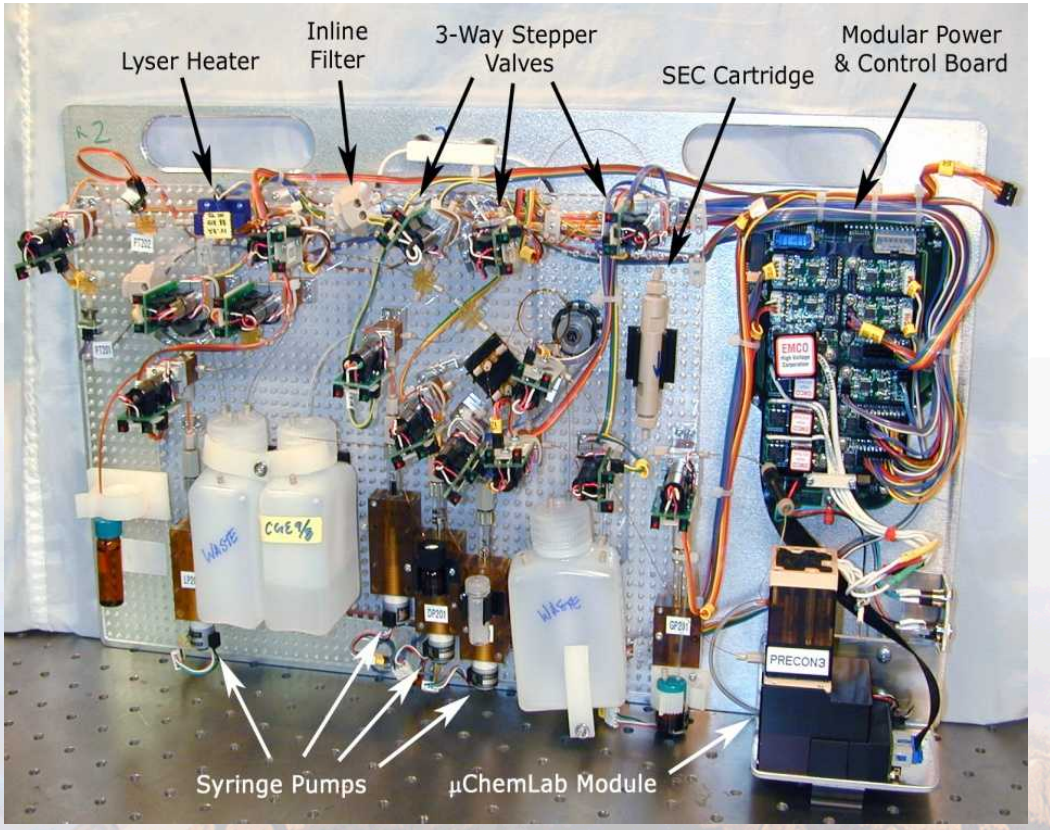
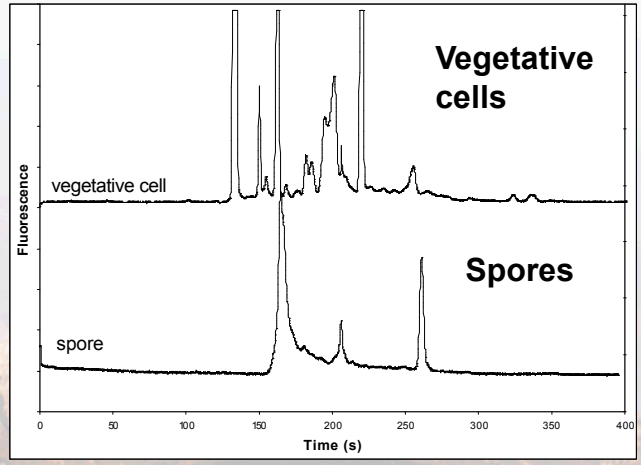
Integrated Biodetection Platforms – Automated Microfluidic Protein Profiling System for bacteria





- **Aerosol point detection**
- **Prototype built and tested**
  - Detects toxins, viruses, spores, and vegetative cells
  - 8 hour autonomous operation
  - Rapid response (minutes)
  - Minimal reagents



Stachowiak *et al, Anal. Chem.* **2007**, 79, 5763-5770

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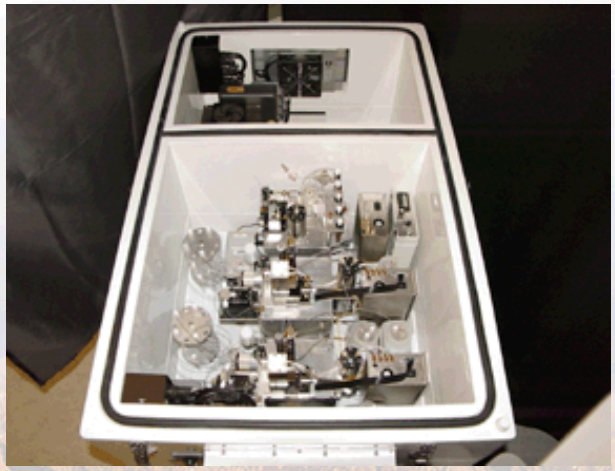
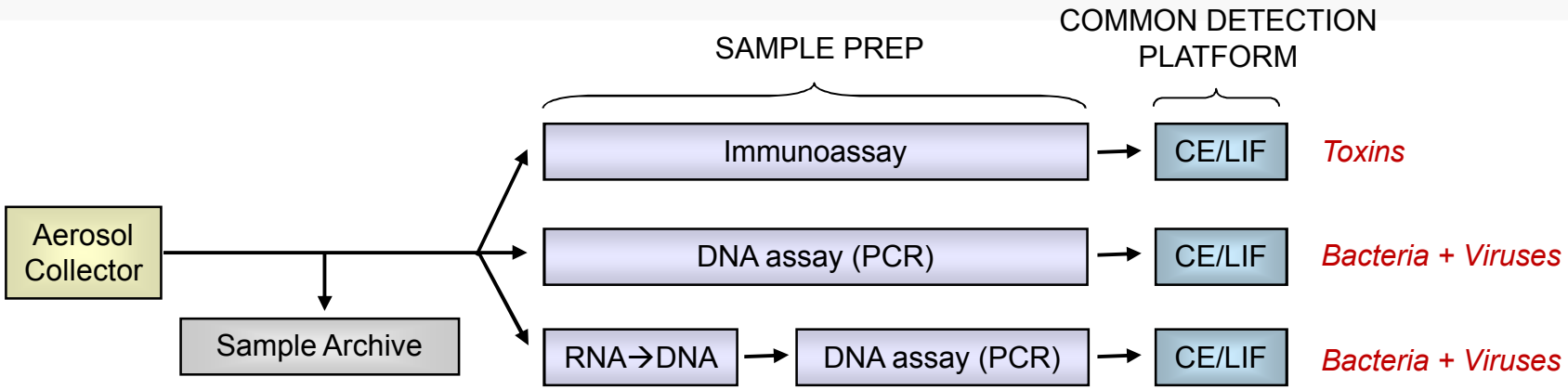
Integrated Biodetection Platforms – BioBriefcase

- **Funded by DOE and DHS**
  - Developed in collaboration with Lawrence Livermore National Laboratories
- **Key system requirements:**
  - Continuous, fully autonomous operation in full range of outdoor environments
  - Broad agent coverage, >20 agents
  - High sensitivity
  - Very low false alarm rate
- **Implemented fluorescent reporter-based assays for PCR and immunoassay detection of bacteria, viruses and toxins**
  - CE/LIF of reporters provides high multiplexing capability

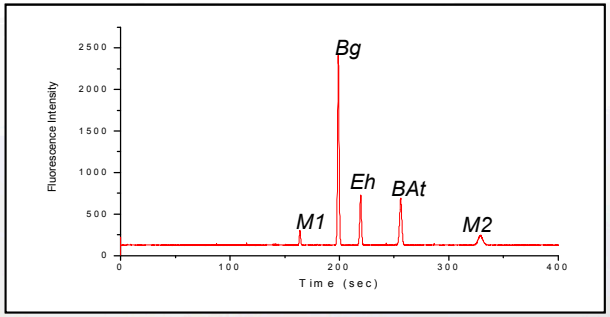


BioBriefcase employs three assay trains to detect the full biothreat spectrum



Assays use "eTags" (small fluorescent reporter molecules) to indicate when a pathogen is detected:

CE/LIF analysis of eTags



Particles in air (1-10 µm in size)

Collector fluid

Liquid sample (1 mL volume)

eTag-labeled antibodies or DNA probes

Binding to targets

Release of eTags

Detect eTags using CE/LIF

Immunoassay: Photolysis
DNA assay: PCR reaction

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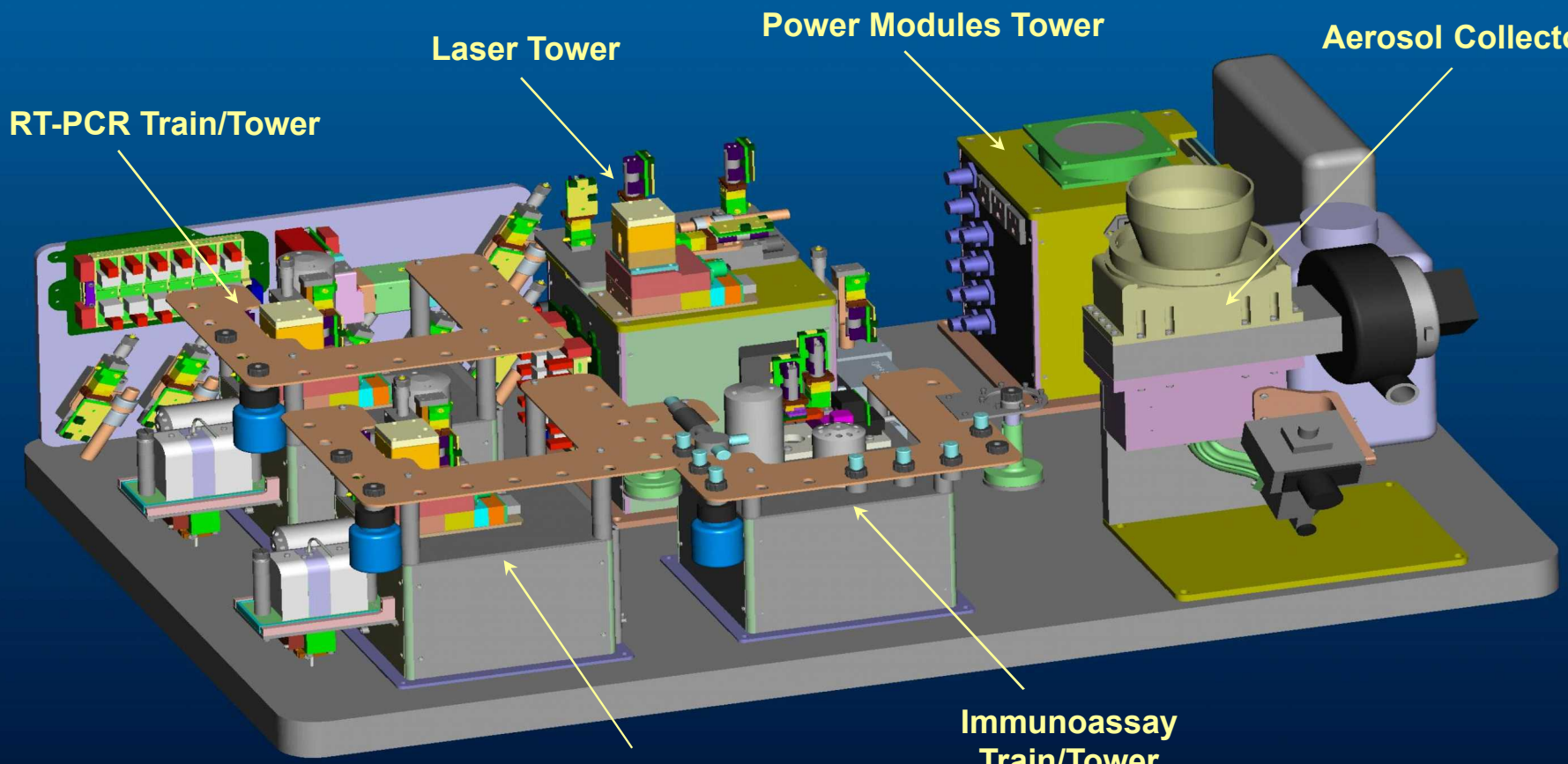
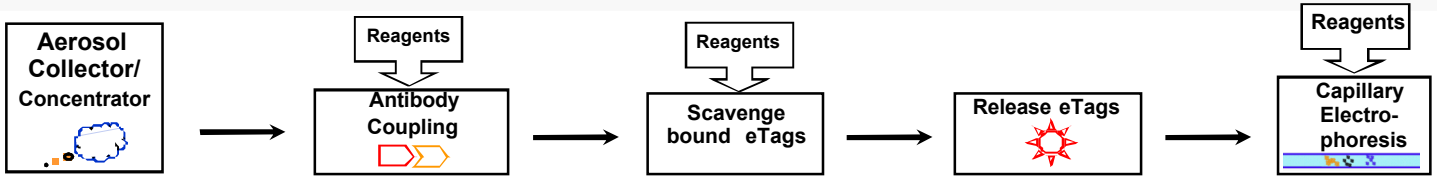
BioBriefcase prototype contains 3 analysis trains, aerosol collector, laser & power distribution towers

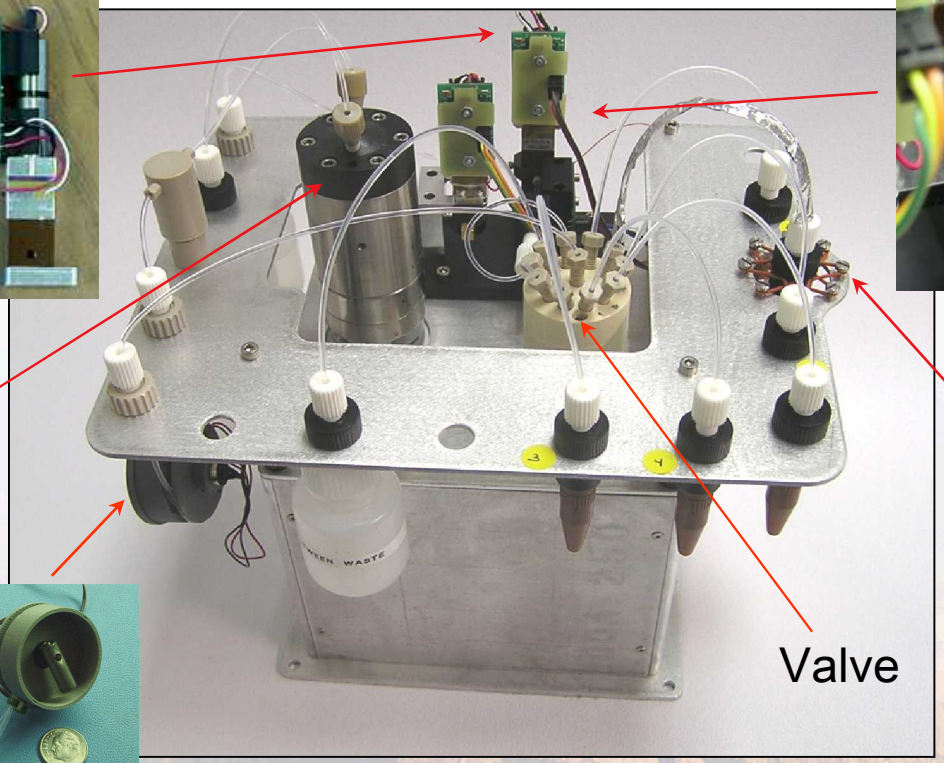


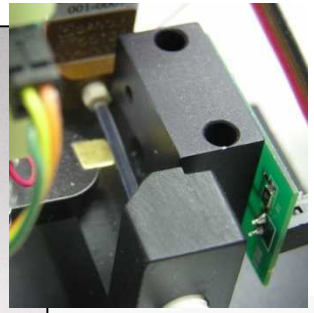
Approximate size is 24 x 36 x 14 inches

Integrated hardware can perform automated bead-based immunoassay



3 way valve < 50 nL dead volume





Magnetic bead trap and eTag release

Pump

Tube bead mixer

Valve

Bead reagent mixer

Kwon *et al, Anal. Chem.* **2008**, 80, 8416–8423

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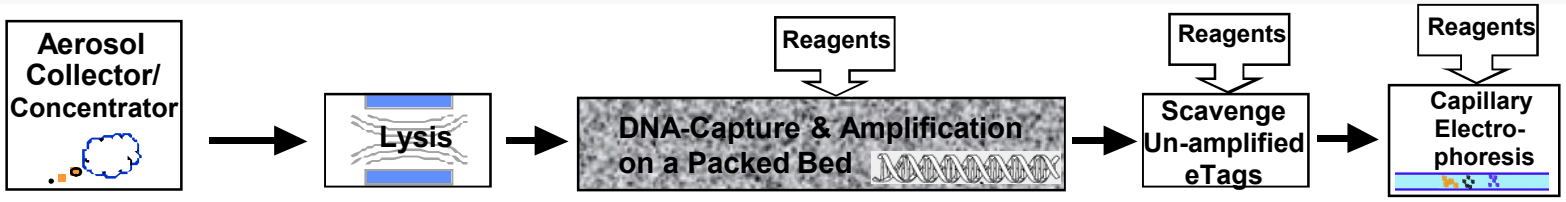
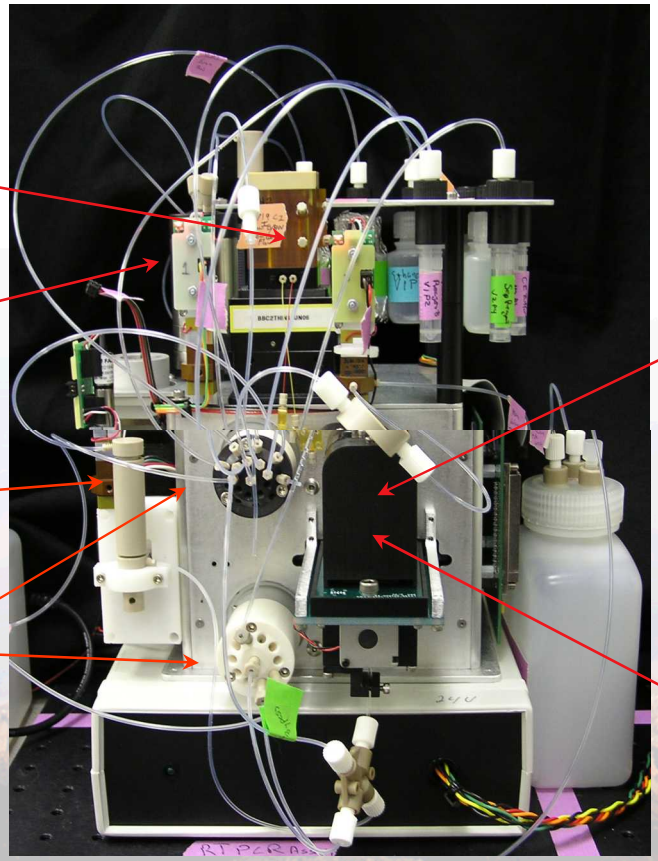
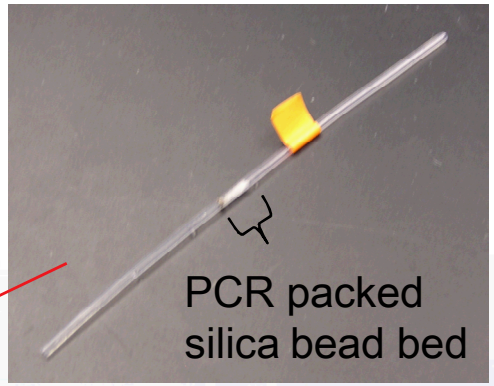
Integrated hardware can perform automated PCR and RT-PCR analyses







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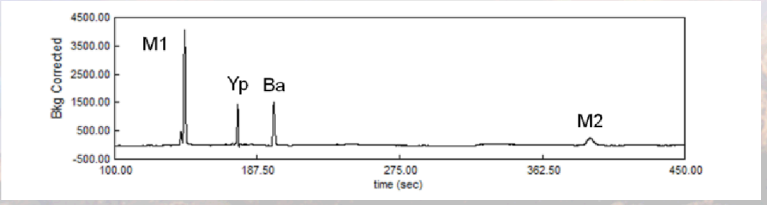
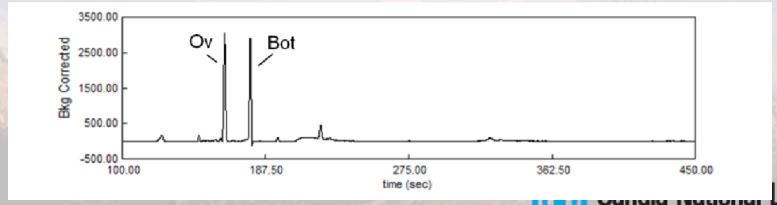
Testing conducted at US Army Edgewood Chemical and Biological Center

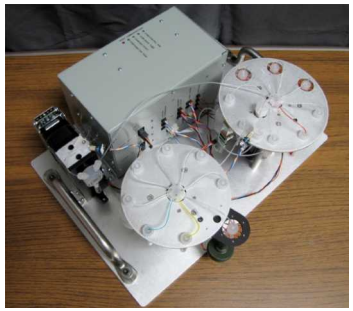
- **Laboratory testing (20 blind samples) –**
  - Liquid sample was drawn automatically into the instrument and analyzed
  - Data analysis software generated the detection calls
- **Aerosol chamber testing (18 blind samples) –**
  - Aerosolized sample was produced directly into the BBC aerosol collector
  - Collection continued for 5 additional minutes, then the collection bowl fluid was pumped into the instrument and analyzed (as above)
- **Summary of results:**
  - Correctly identified 87% of samples over the two series of blind challenge tests
    - Reasons for incorrect calls were immediately identified and correctable
  - Although ambient temperature variations shifted CE peak times, we could account this for using internal standards
    - Independent study of temperature effects indicates a minimum of two internal standards are required for accurate peak calling
    - Fielded instrument will be environmentally controlled





Conclusions

Next gen fluidic hardware



• Comparison of a commercial valve (left) and Sandia 10-port valve (right)
• The commercial valve is 5 times larger, 20 times heavier, and requires 40 times more power to operate than the Sandia valve

- **We have demonstrated capability to successfully build and test portable biodetection instruments using miniaturized assays and componentry**
- **Our modular design approach enables rapid prototyping of instruments and flexibility to meet new user needs**
- **By miniaturizing robust analytical methods and instrumentation, system performance is competitive with benchtop systems while meeting field portability requirements**
- **We are currently extending this technology into medical diagnostics, bioanalysis of precious samples and other areas including biodetection, and welcome opportunities to collaborate**