SAND2007-0778P

BioBriefcase

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This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

BioBriefcase is a collaboration between Lawrence Livermore and Sandia National Laboratories

Objective – to develop a portable bioagent detection system for national security applications

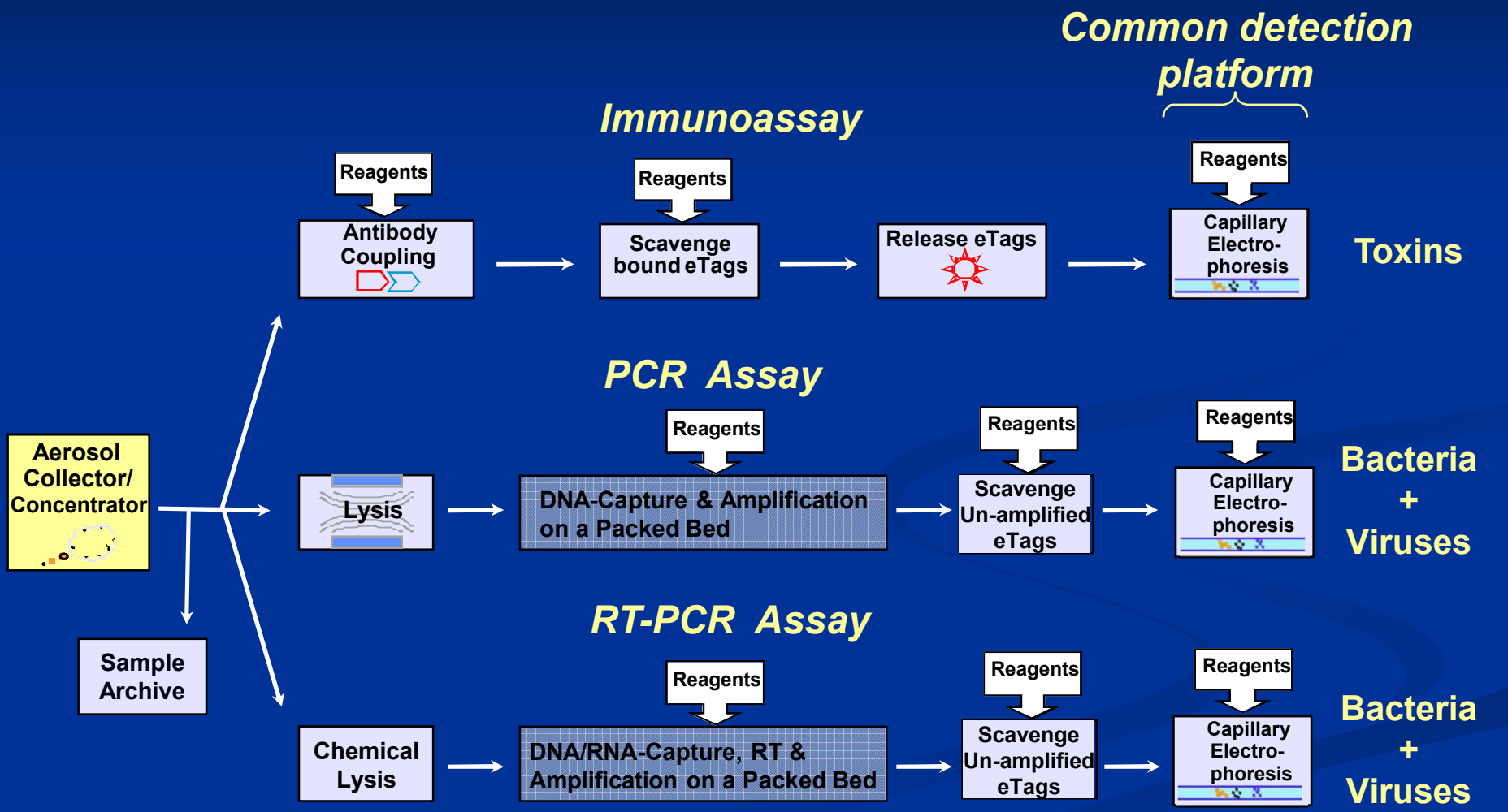
- **Operations -**
  - Autonomous
  - 30 days between servicing
- **Costs -**
  - Inexpensive to own and operate
  - Low reagent consumption
- **Assays -**
  - Multiplex analysis
  - Parallel detection trains
- **Deployment -**
  - Portable (< $2ft^3$)
  - Easily deployed and networked
  - Monitor public facilities and special events

2003 – DOE/CBNP funding

2004 – DHS ORD funding

2006 – DHS HSARPA funding

BioBriefcase employs three analysis trains to detect the full biothreat spectrum

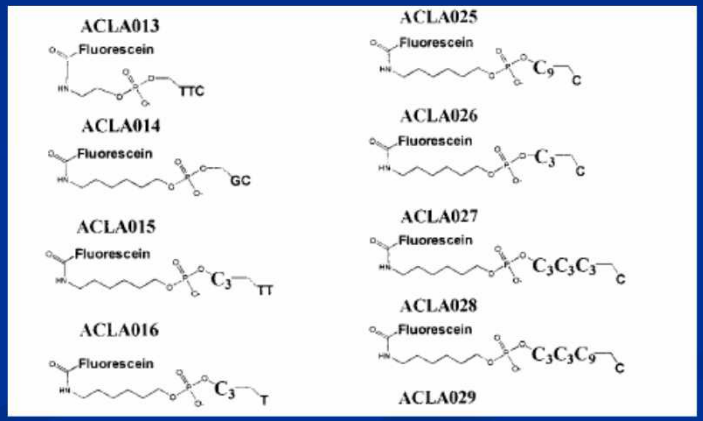


BioBriefcase uses eTag-based assays for both immuno and nucleic acid assays

eTag assays were developed for medical diagnostics by Monogram Biosciences, customized for BioBriefcase for bioagent detection

Reporter Molecules –

- Decouple recognition from detection
- Optimized conditions for each step
- **Enables multiplex detection**
- **Multiple loci and antibodies for a single agent**

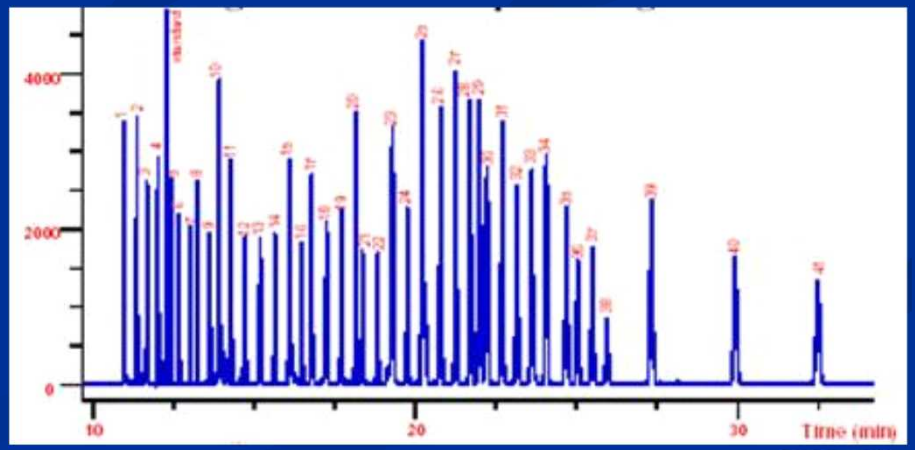


Monogram eTags -

- Organic molecules 500-1000 MW
- Well characterized electrophoretic mobility
- Tagged with same fluorophore

CE Analysis -

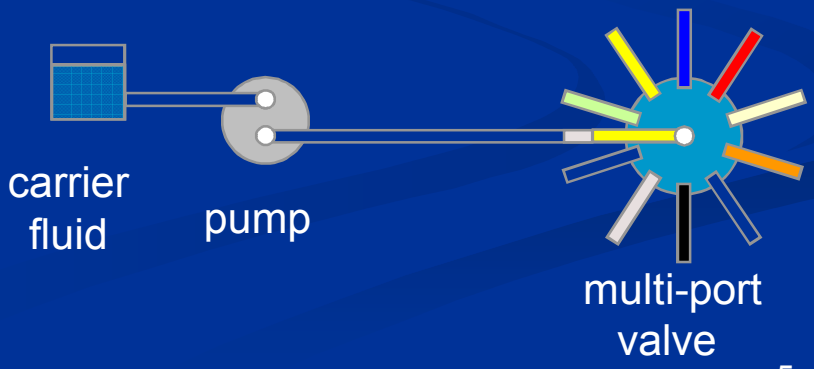
- Amenable to analysis on chip
- Rapid separations



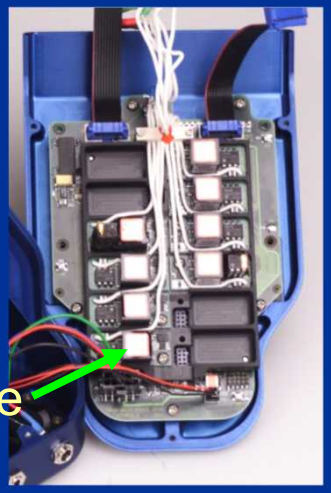
Zone fluidics – automated approach for manipulating fluids for chemical processes

- Can perform most standard unit operations
- Simple components – reversible pump, multi-port selection valve, versatile control software
- Air bubbles are used to separate reagents and minimize diffusion
- Compatible with microfluidics

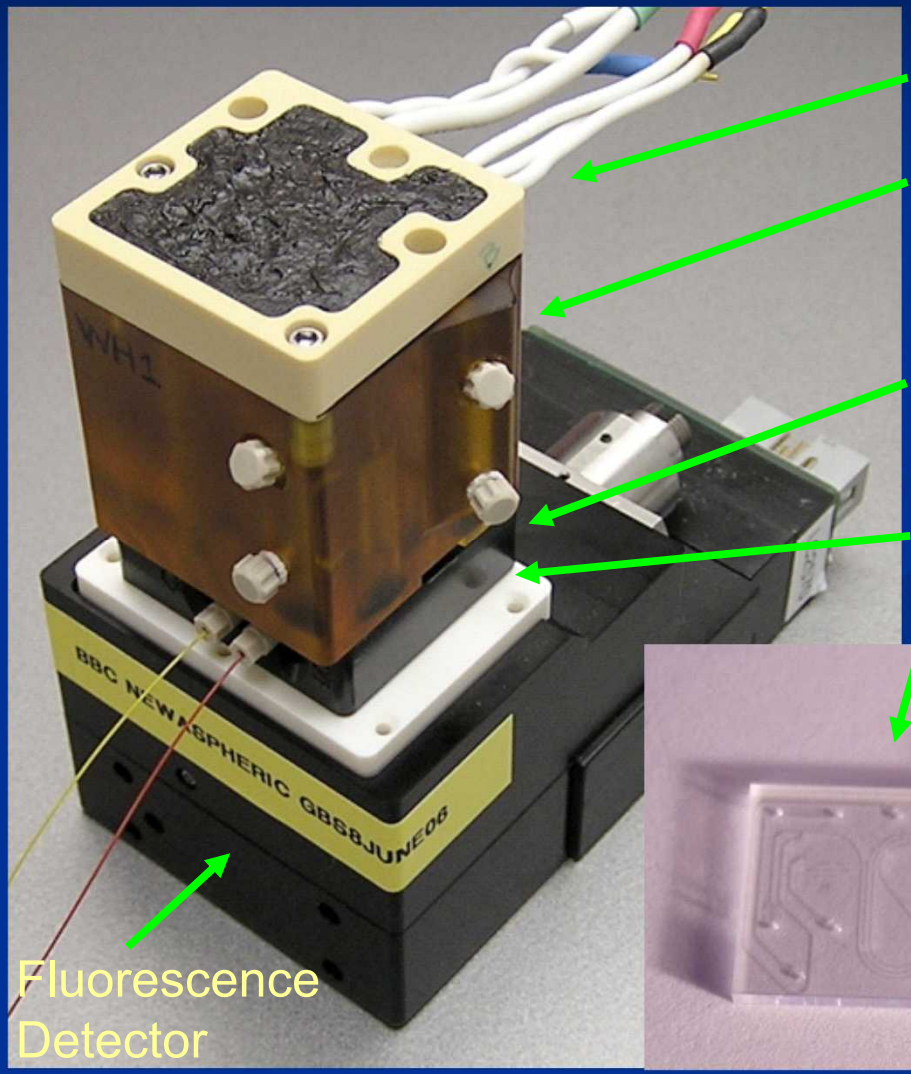
Global FIA mini FloPro

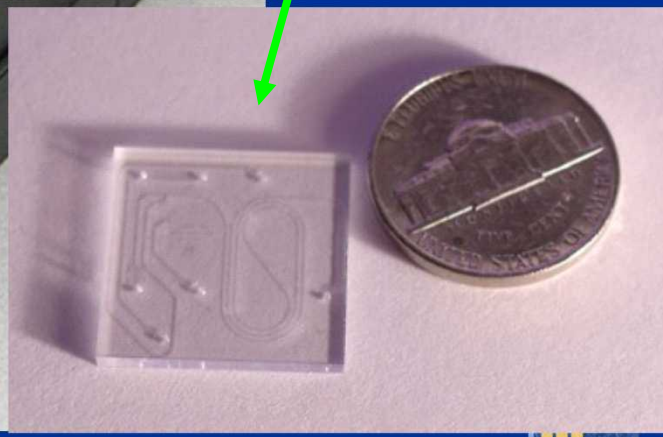


Sandia's µChemLab™ hand-held instrument provides the CE/LIF detection platform for BioBriefcase



High-Voltage Power Supplies

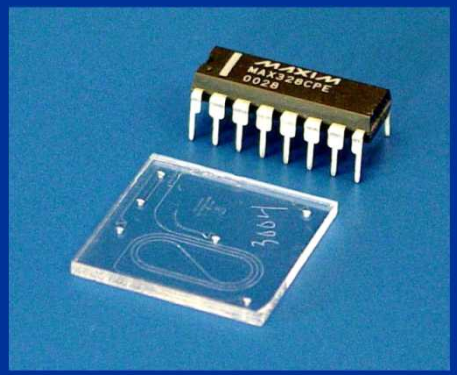




CE analysis performed on-chip for rapid (5 min), compact analysis

Electrokinetic injection

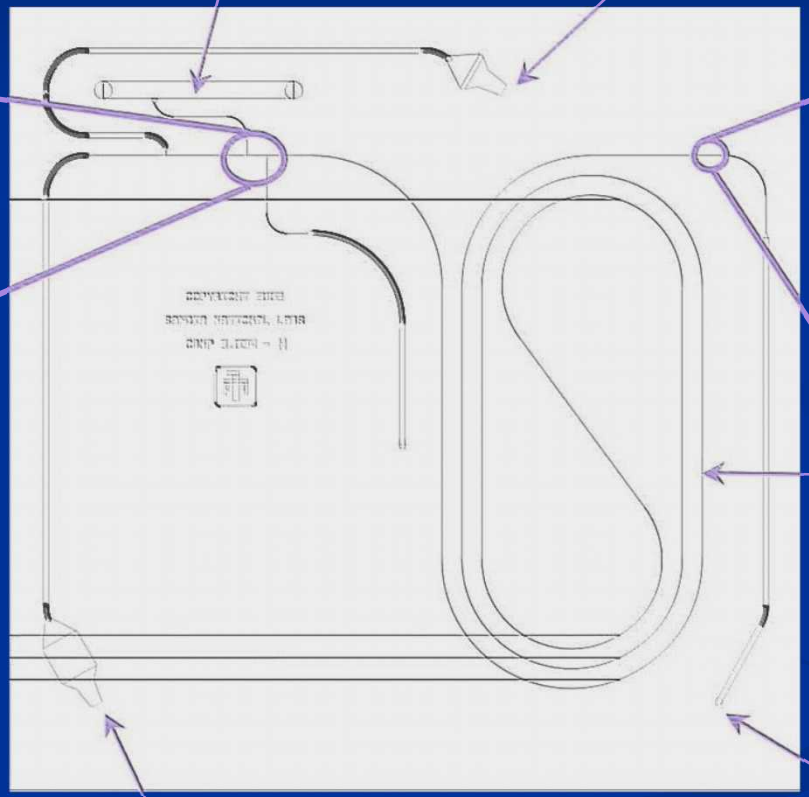
Sample plug



Overall 20 x 20 mm

Sample Loop

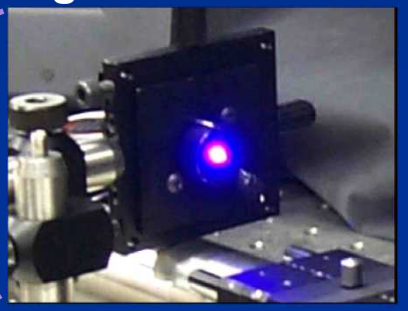
Flush port



Column Head

Column Waste

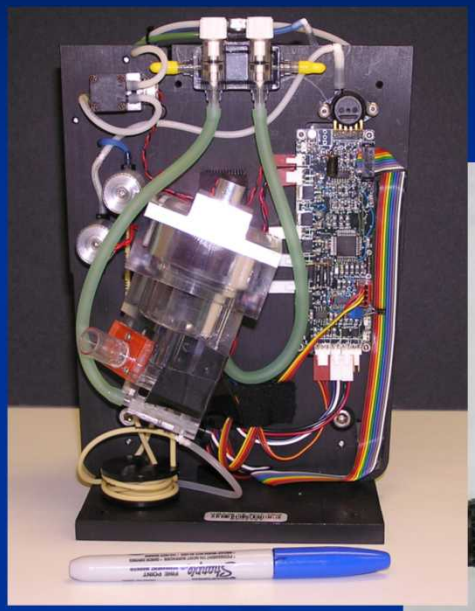
LIF detection region



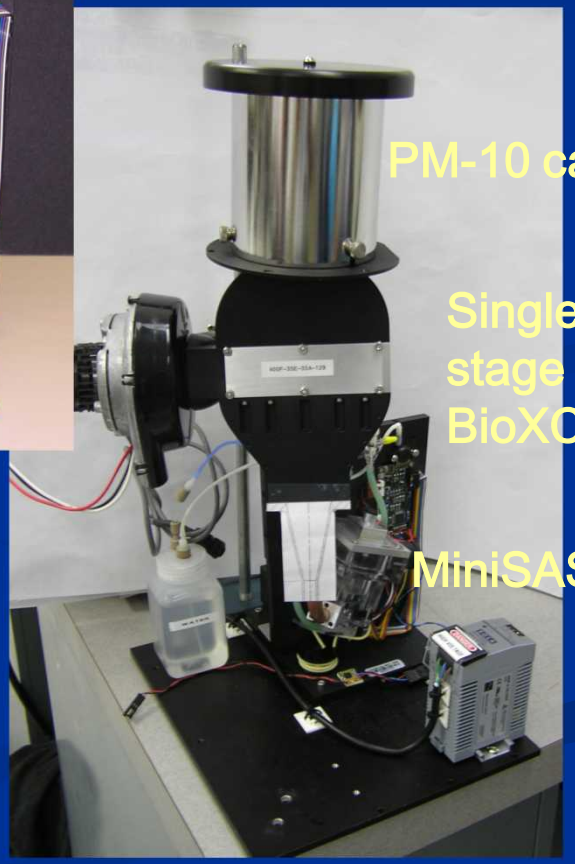
Separation column, 10 cm length, 500 V/cm

Aerosol collector draws in 400 L/min, deposits particles in 1 mL fluid

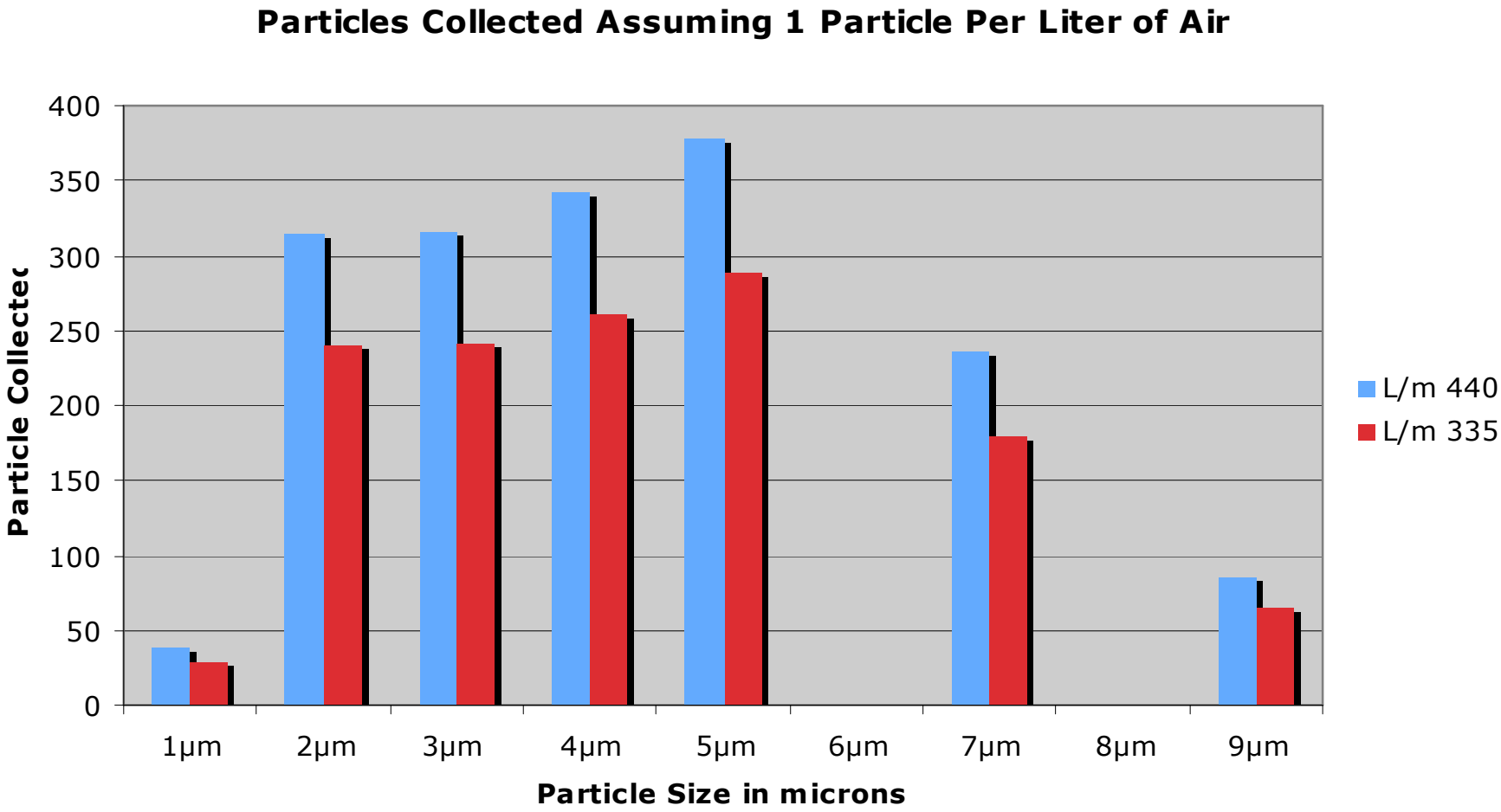
- Research International MiniSASS
  - Wetted wall cyclone
  - 30 – 50 L/min
  - 1 mL sample
  - ~8 L/month water
- MesoSystems BioXC Aerosol Concentrator
  - First stage concentrates from 400 to 33 L/min
  - Second stage from 33 to 3 L/min



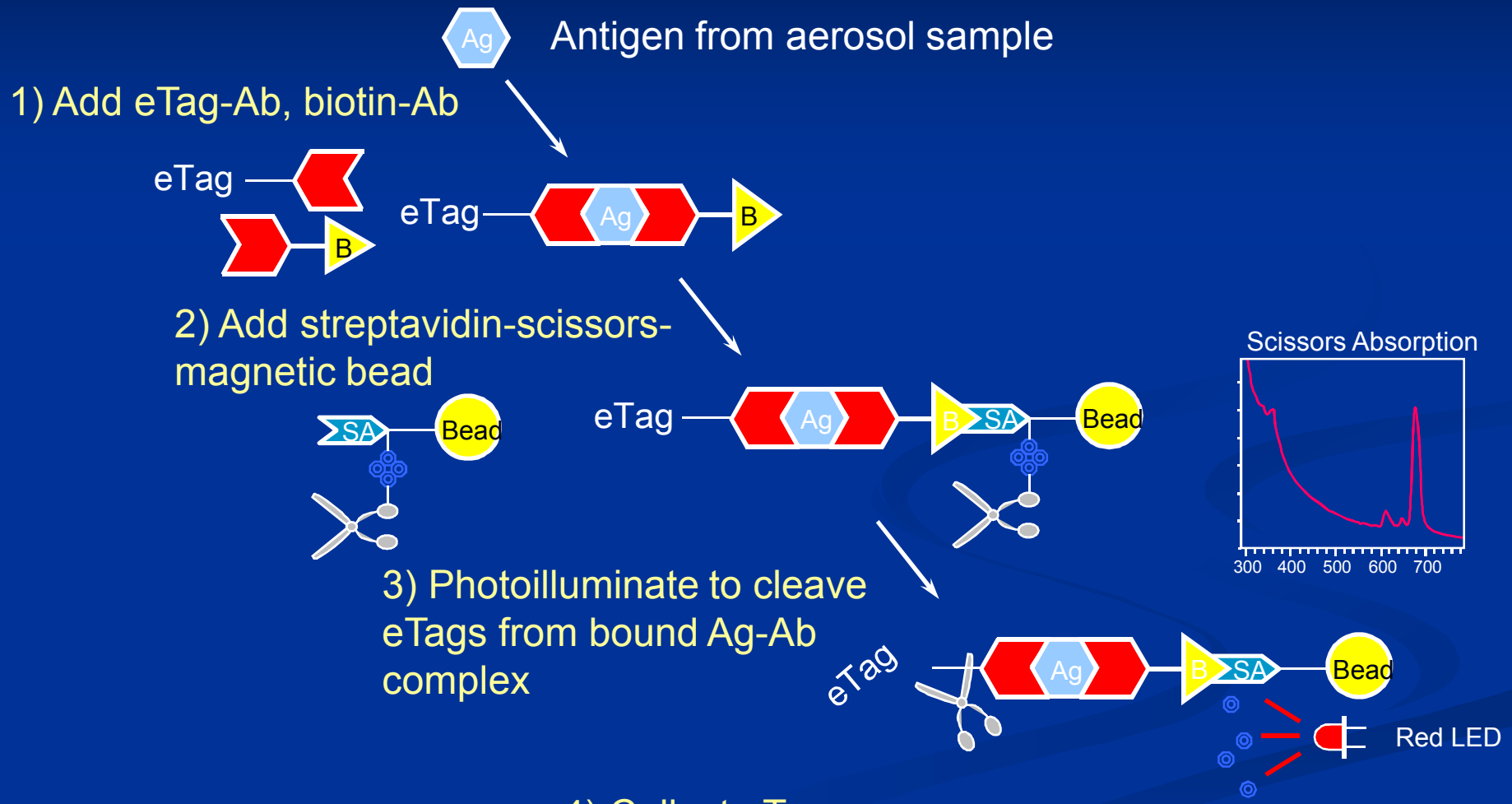
MiniSASS



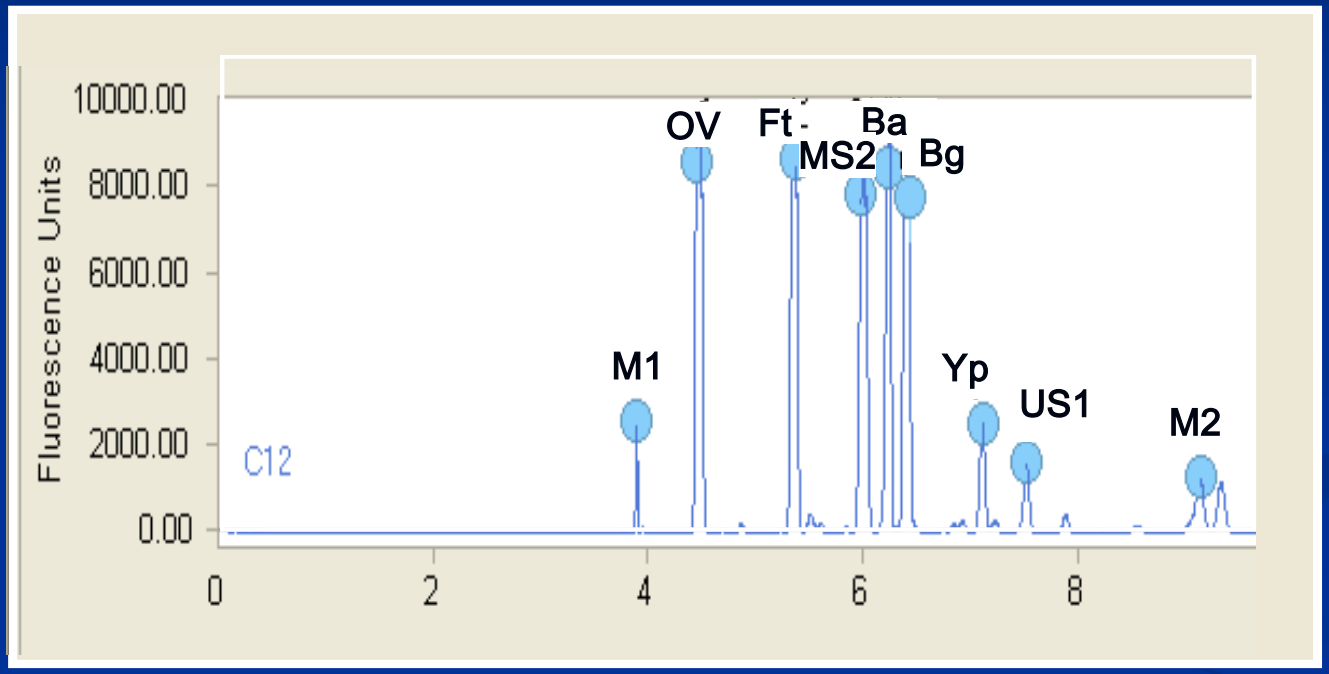
Collector demonstrated ~70% efficiency during aerosol chamber tests



eTag reporter immunoassay for toxins



eTag immunoassay offers high multiplexing capability

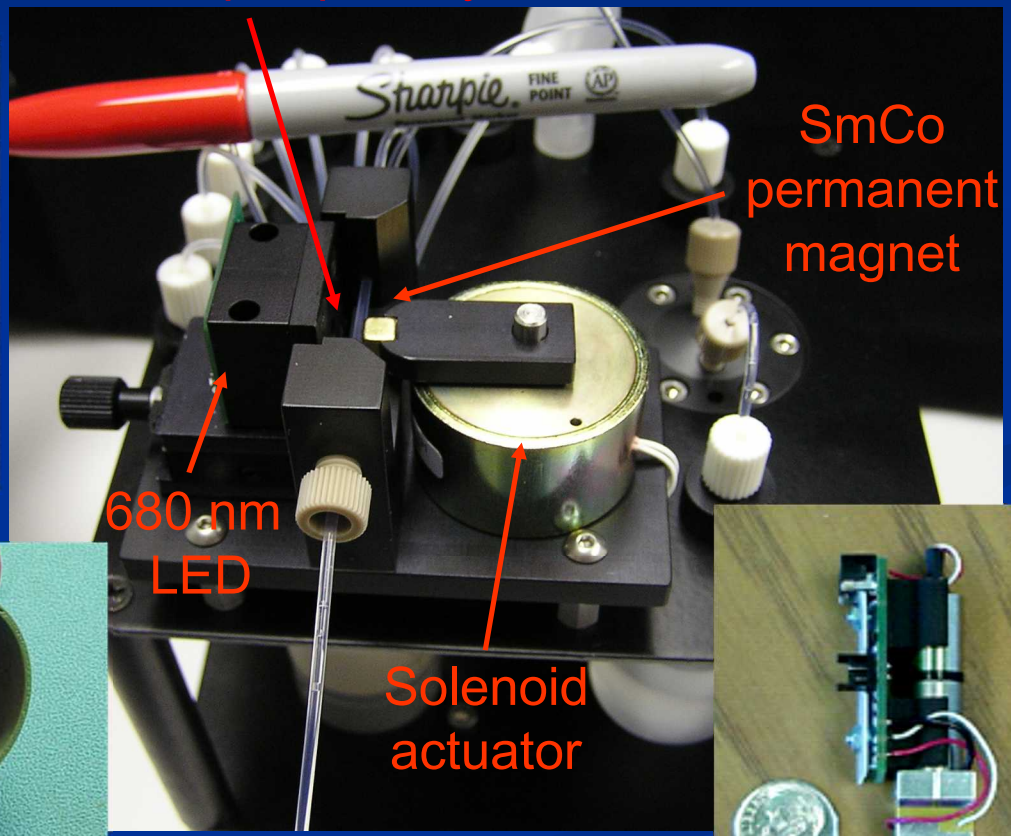


Time and quantification control markers are included

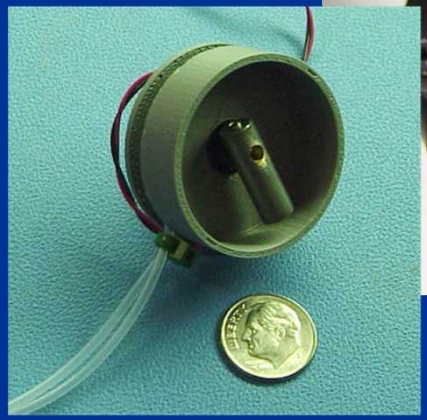
Flow-through IA platform incorporates custom hardware for magnetic bead mixing & trapping

Bead trap & photolysis

Bead reagent mixer

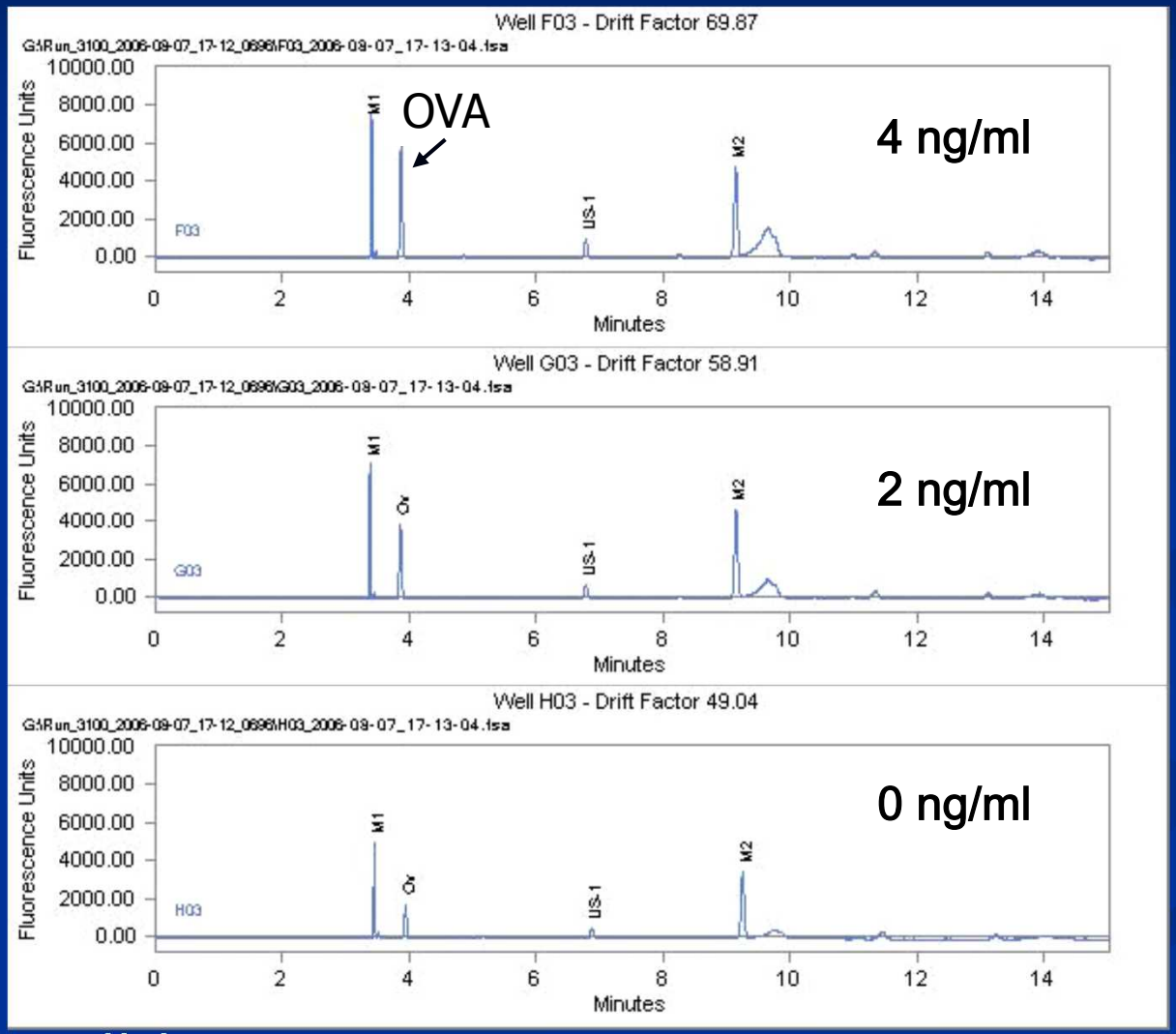


Tube bead mixer

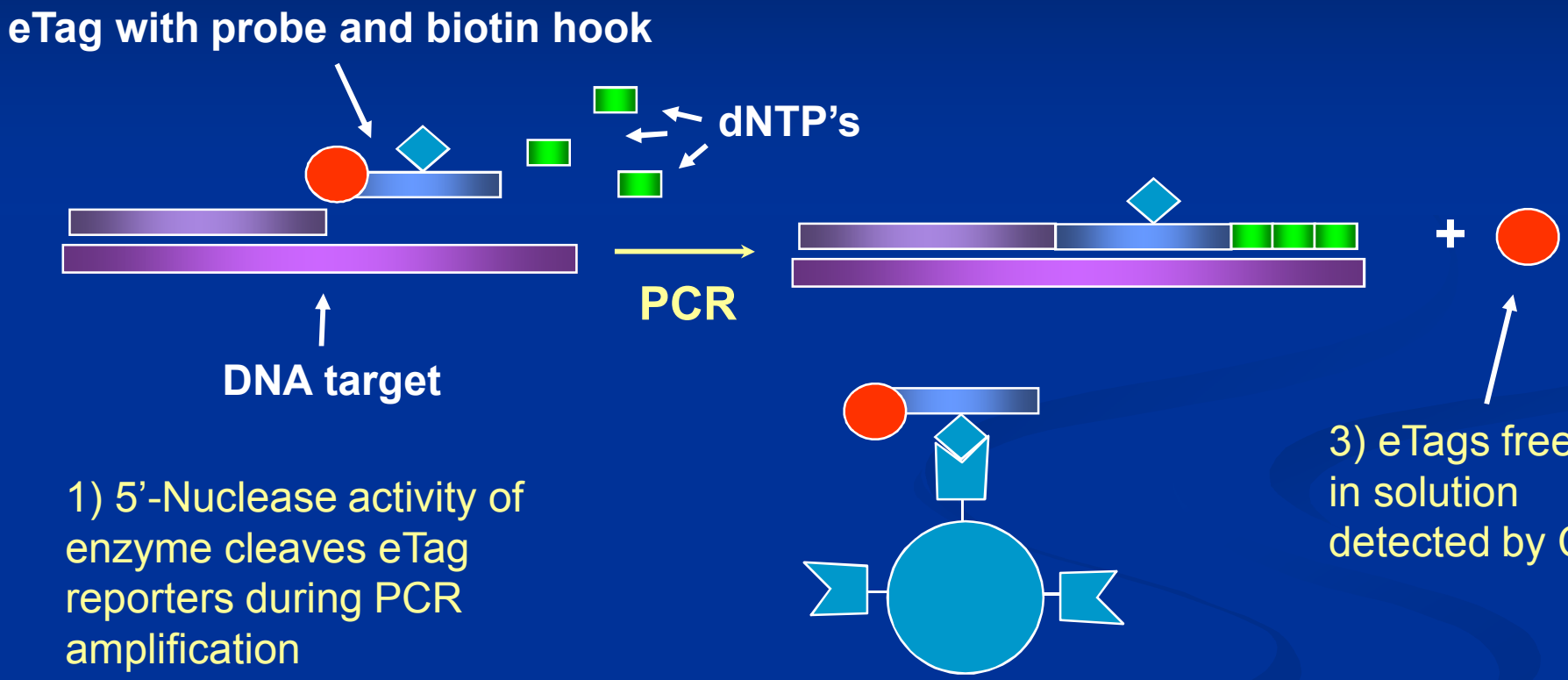


3-Way valve (dead volume < 50 nL)

Optimized immunoassay reactions show excellent sensitivity for ovalbumin



eTag reporter nucleic acid assay for bacteria and viruses

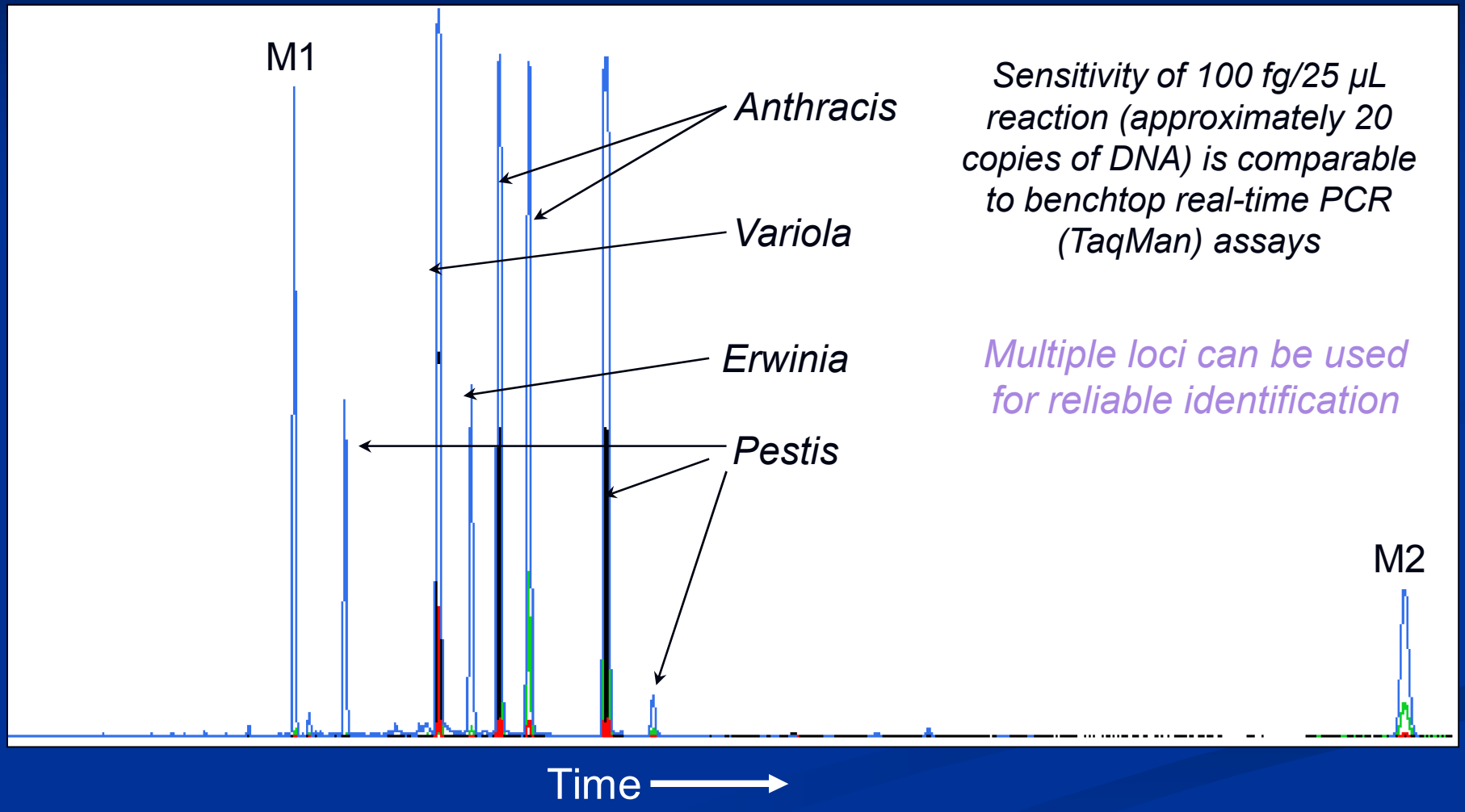


1) 5'-Nuclease activity of enzyme cleaves eTag reporters during PCR amplification

2) Streptavidin scavenges free eTag-labeled primers

3) eTags free in solution detected by CE

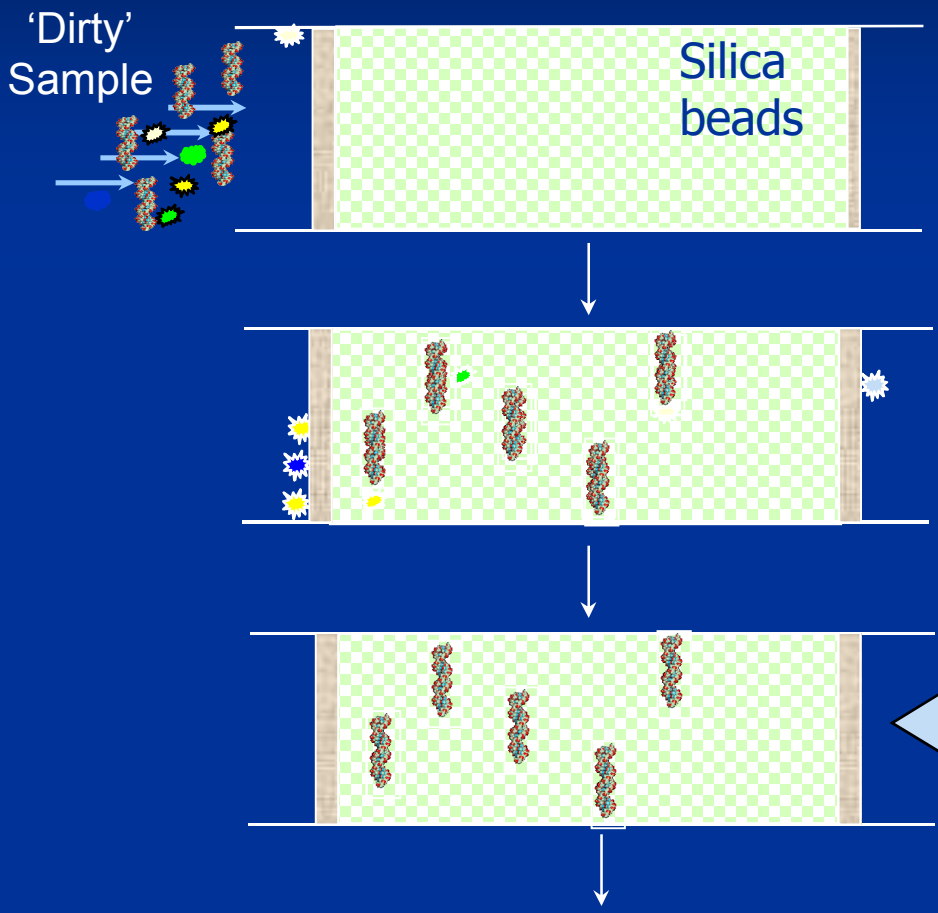
eTag nucleic acid assay shows high multiplexing capability using flow-through thermal cycler



Sensitivity of 100 fg/25 µL reaction (approximately 20 copies of DNA) is comparable to benchtop real-time PCR (TaqMan) assays

Multiple loci can be used for reliable identification

DNA purification and concentration on a packed bed minimizes losses



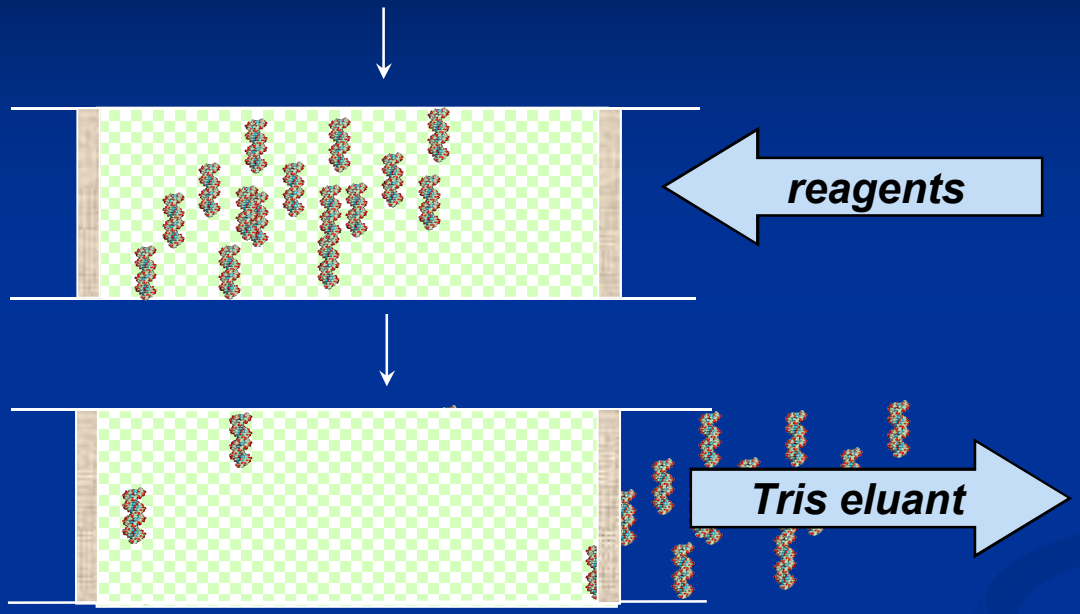
Dirty DNA sample is mixed with chaotropic salt and passed through a packed bed of silica beads

DNA binds to silica beads

Inhibitors are washed away

Standard columns elute DNA at this step, resulting in ~60% of DNA remaining bound to beads at low DNA masses

Amplifying DNA bound to silica beads improves sensitivity

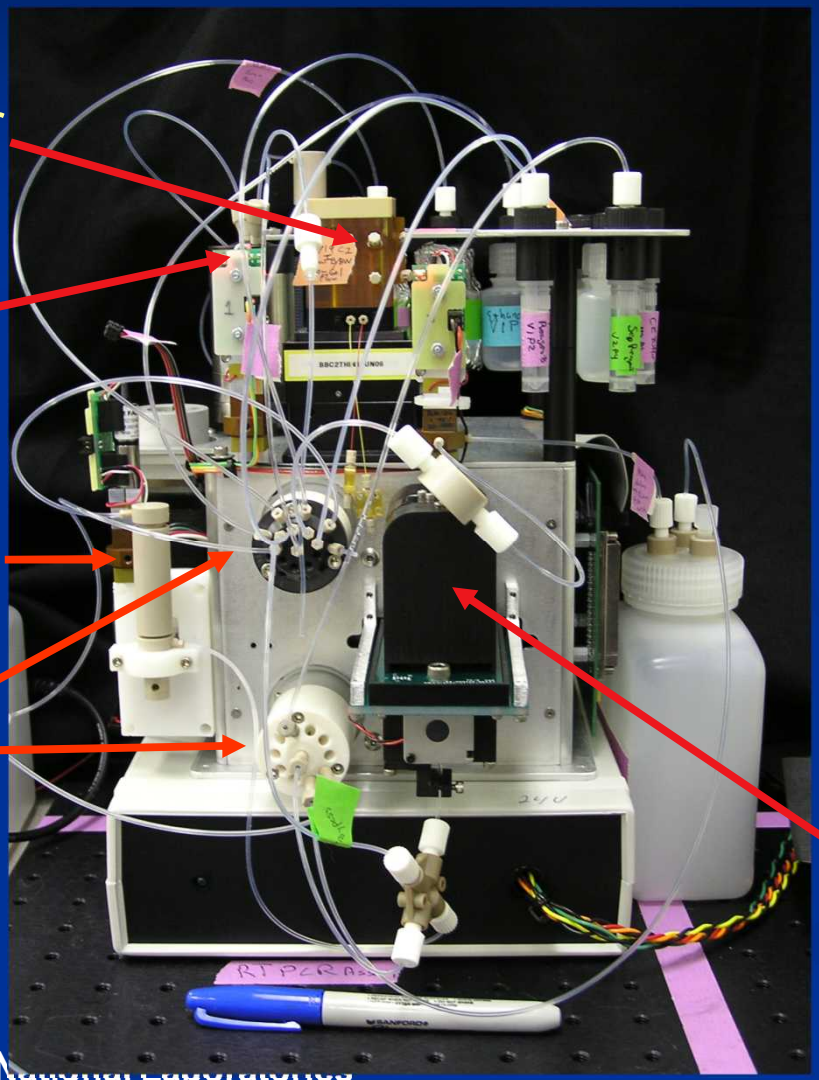


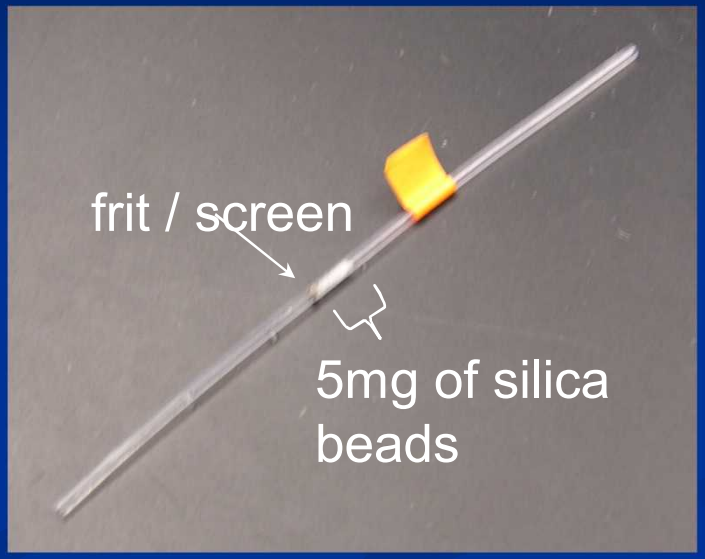
Amplification occurs on bound DNA; DNA is not eluted from surface, reducing losses

Amplified DNA is eluted for analysis

	% successful PCR	
Amount of DNA (ng)	Within column	Eluted from column
2	100	100
0.5	100	100
0.05	100	20
0.02	100	0

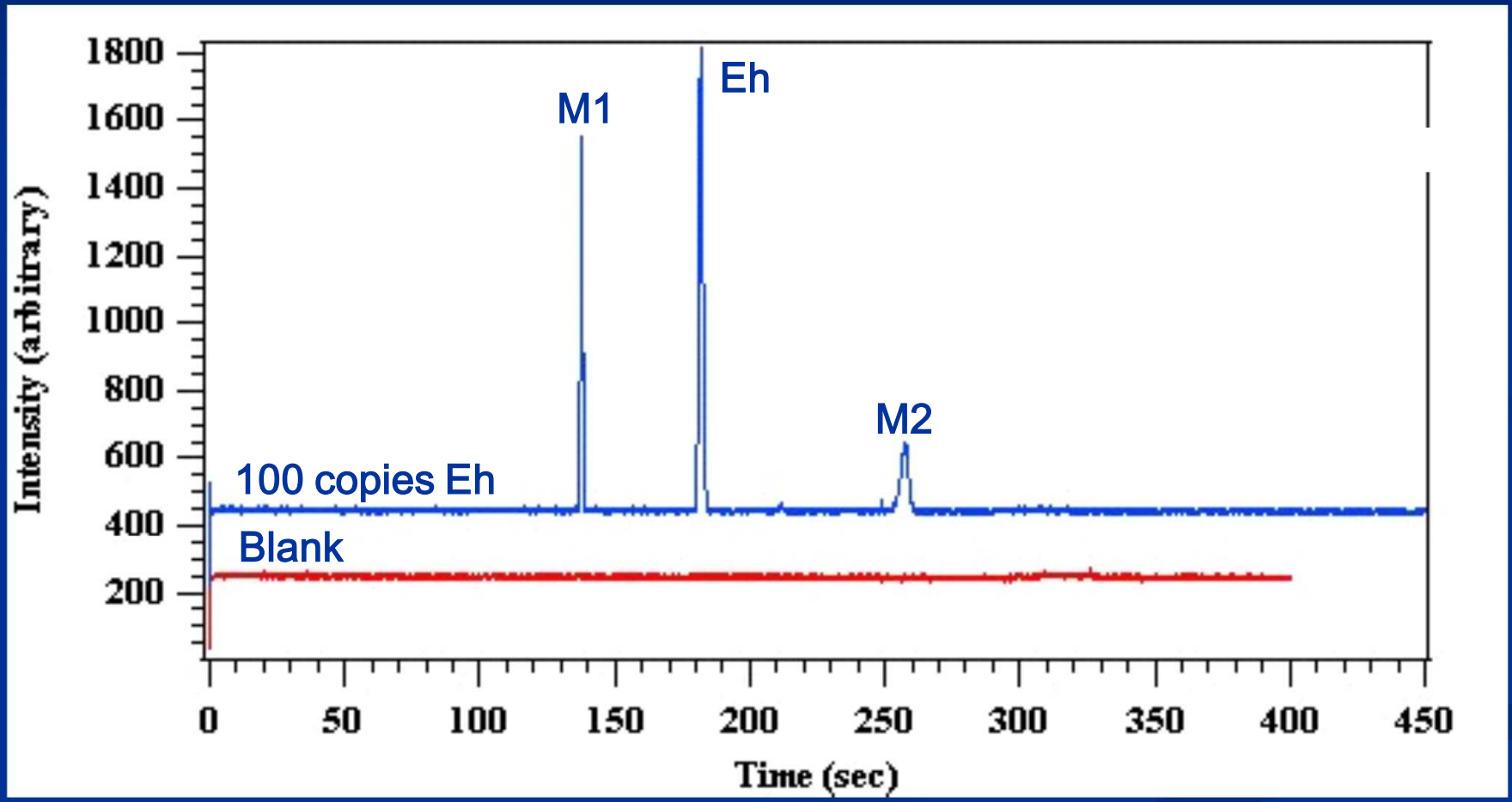
Nucleic acid assay platform incorporates lysis with flow-through PCR amplification



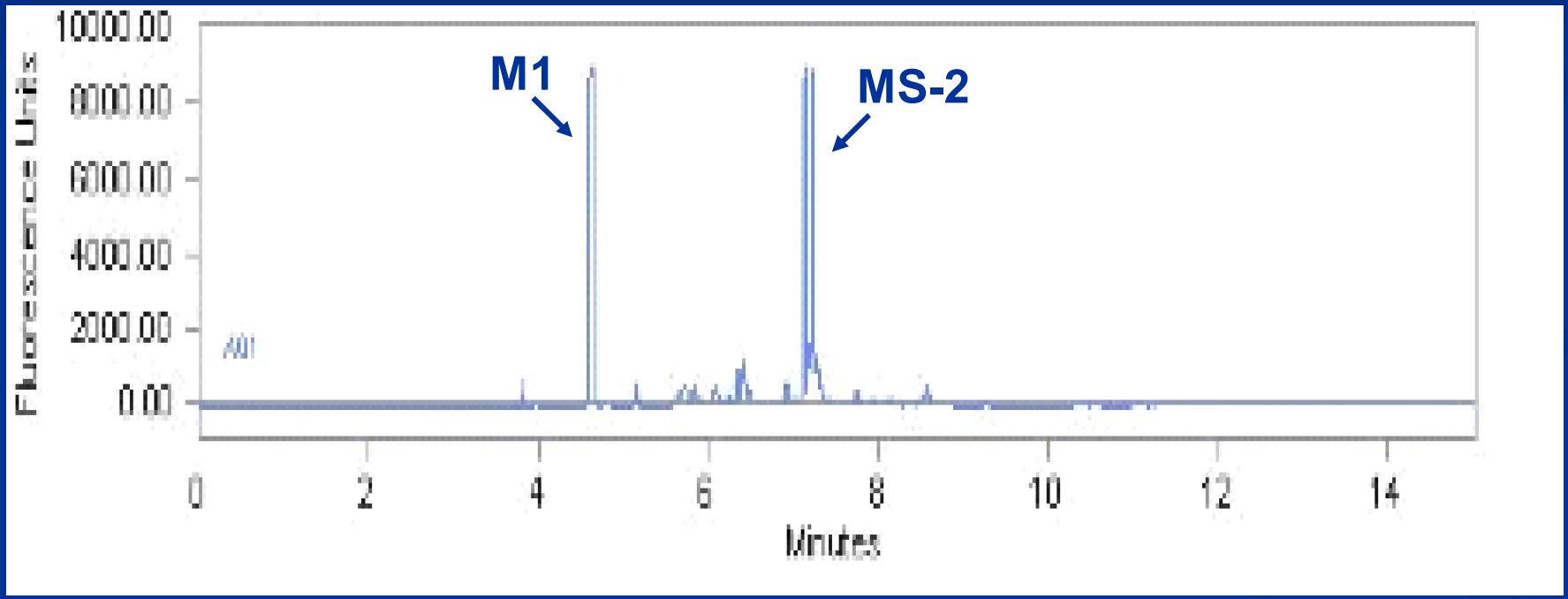


PCR packed bed

eTag nucleic acid assay shows excellent sensitivity for bacterial DNA detection



Addition of reverse-transcriptase step prior to PCR amplification allows detection of RNA viruses



RNA-virus simulant MS-2 successfully demonstrated using flow-through platform

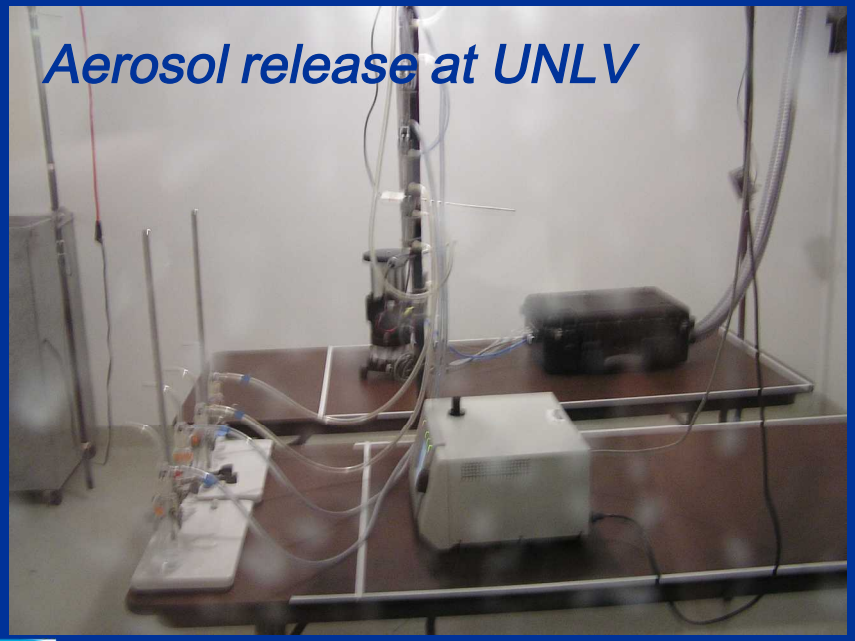
Biobriefcase laboratory prototypes have undergone testing at two venues

- Early prototype – Aerosol chamber tests at the Harry Reid Center for Environmental Studies at University of Nevada, Las Vegas
  - Under DHS ORD funding
  - Objectives were to:
    - Test and select among 3 aerosol collectors
    - Test assays and analysis trains using aerosol samples
    - Evaluate performance of components, coupling of components and integrated analysis trains
    - Prepare for 2006 BAND testing
- BAND prototype – Laboratory and aerosol chamber tests at the US Army Edgewood Chemical and Biological Center
  - Part of the DHS Bioagent Autonomous Networked Detector (BAND) Phase II program

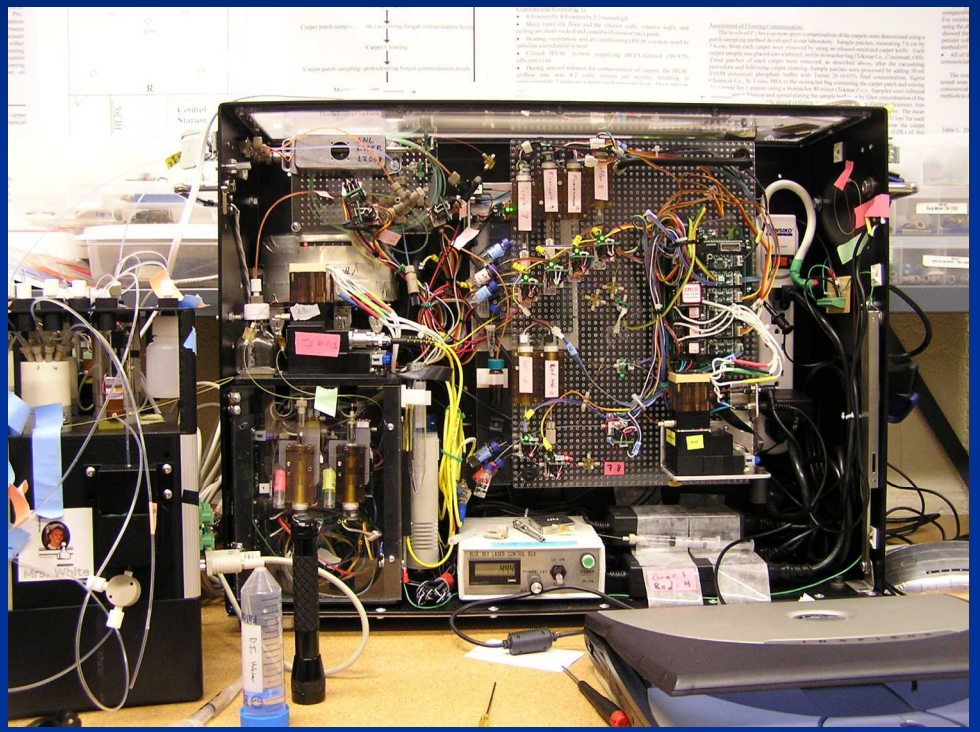
UNLV environmental chamber testing

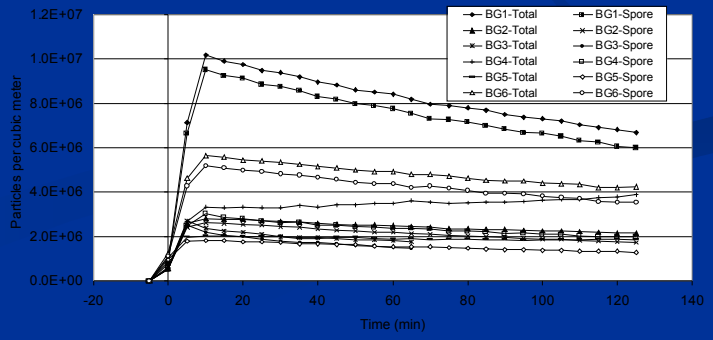
- Aerosol releases of *Bacillus globigii, Erwinia herbicola* and ovalbumin were collected
- Samples were transferred to the three analysis trains for processing

Aerosol release at UNLV



BioBriefcase outside the chamber





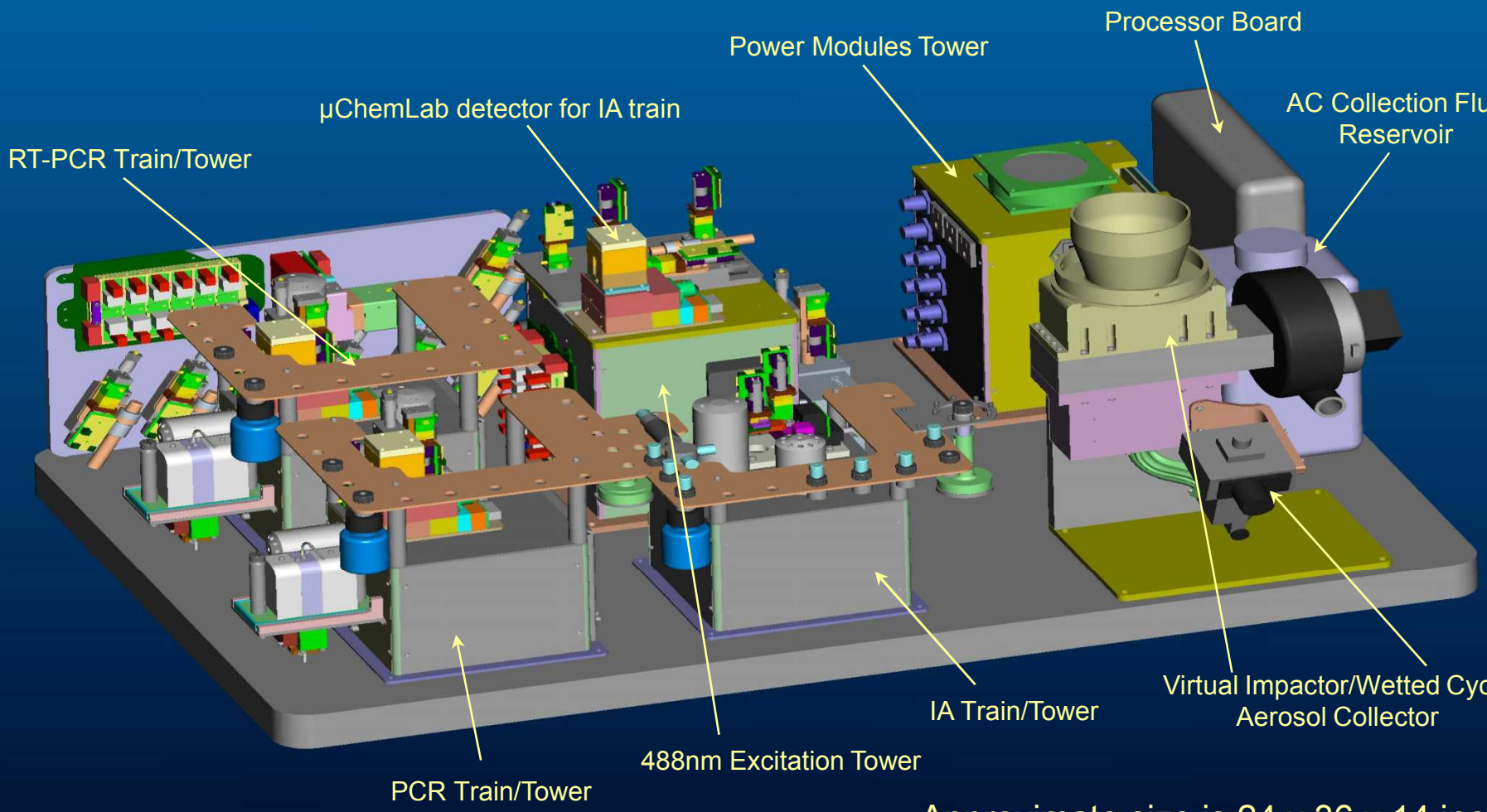
UNLV test results summary

- We saw positive results from all of the aerosol collectors
  - Performance of the Mini-SASS at these tests was critical for collector downselect from 3 candidate technologies
- We detected each of the released species: spores, vegetative cells, toxin simulant
- We demonstrated partial to full integration with each of the trains

DHS Bioagent Autonomous Networked Detector program performance goals

- Performance Targets
  - Continuous, fully autonomous operation
  - Broad agent coverage > 20 agents
  - High sensitivity – Limit of detection of 100 organisms (10 ng toxin)
  - Single agent false positive rate of 10^{-7} with a goal of 10^{-8}
- Cost of Ownership
  - Unit acquisition cost of $25K per unit or less (quant. > 1000's)
  - Operating costs per unit of $10K per year or less inclusive
- System Requirements
  - Preserve samples for 5 days for confirmation and forensics
  - Robust wireless, autonomous remote operation
  - Maintenance interval exceeding 1 month
  - Operation in full range of outdoor environments
  - Modest packaging and logistical requirements (i.e. 2 ft^3)

BioBriefcase BAND prototype containing 3 analysis trains, aerosol collector, laser & power distribution tower



Approximate size is 24 x 36 x 14 inches

BAND Phase II testing

- Test 2 – Aerosol collector performance
- Test 3 – Analyzer only (no aerosol collector), spores
- Test 4 – Aerosol collector + analyzer, spores
- Test 5 – Analyzer only for larger test suite, including toxins, vegetative bacteria and RNA viruses

Acknowledgments

Sandia National Laboratories

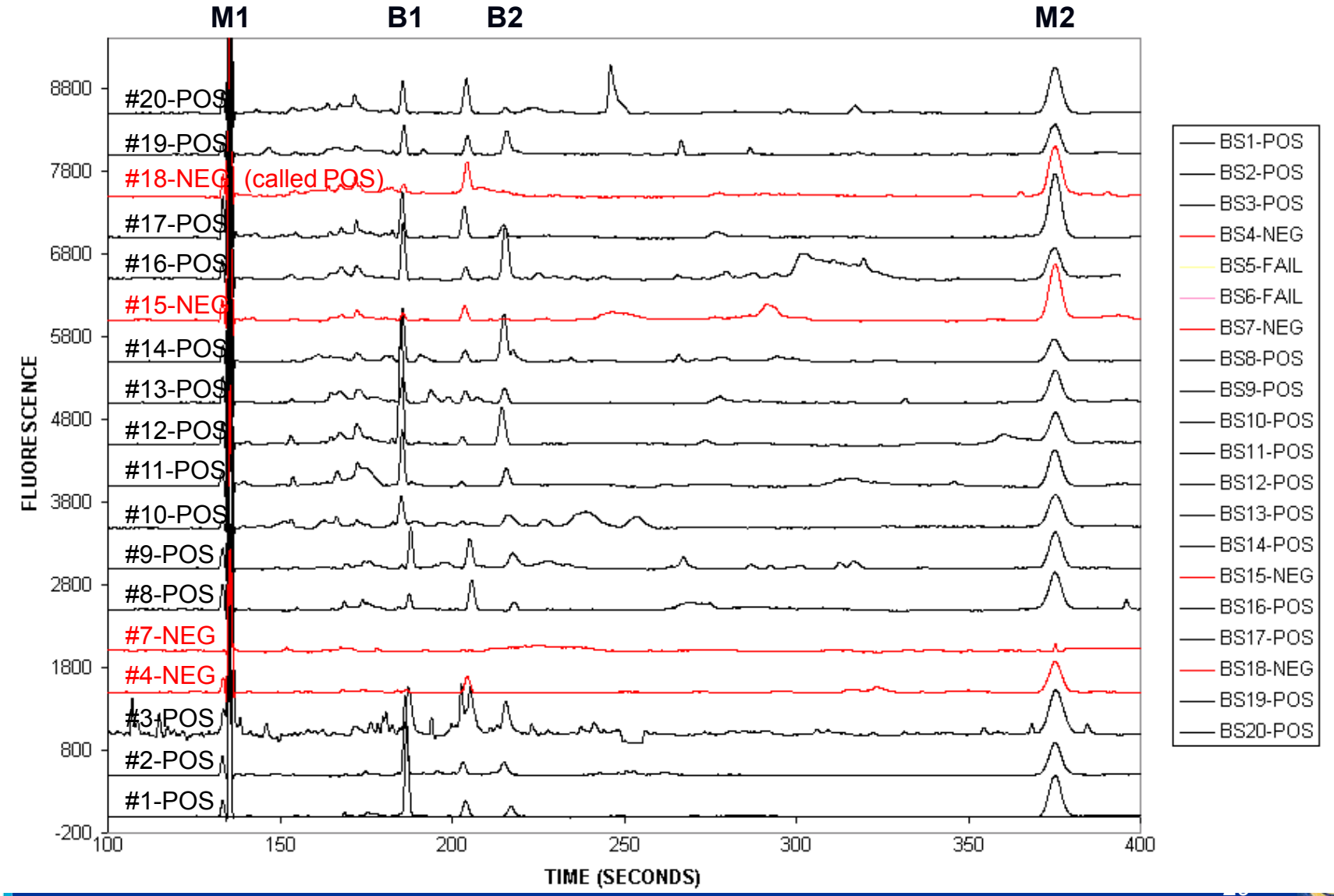
Julie Fruetel, Ron Renzi, Bruce Mosier, Jim Van de Vreugde, Brent Haroldsen, Alf Morales, Shane Sickafoose, Robert Crocker, Tom Raber, Dan Yee, George Sartor, Mark Claudnic,Jerry Inman, Karen Krafcik, Jimmy Ross, Mary Clare Stoddard

Lawrence Livermore National Laboratory

Christopher Bailey, Perry Bell, Elizabeth Wheeler, Anne Erler, Kirsten Johnson, Sean McNary, Bruce Henderer, Olgica Bakajin, Christine Hara, Mona Hwang, Shanavaz Naserabadi, Youngeun Kwon, Paul Butler, Robert Costa, Kristen Maitland

Extras

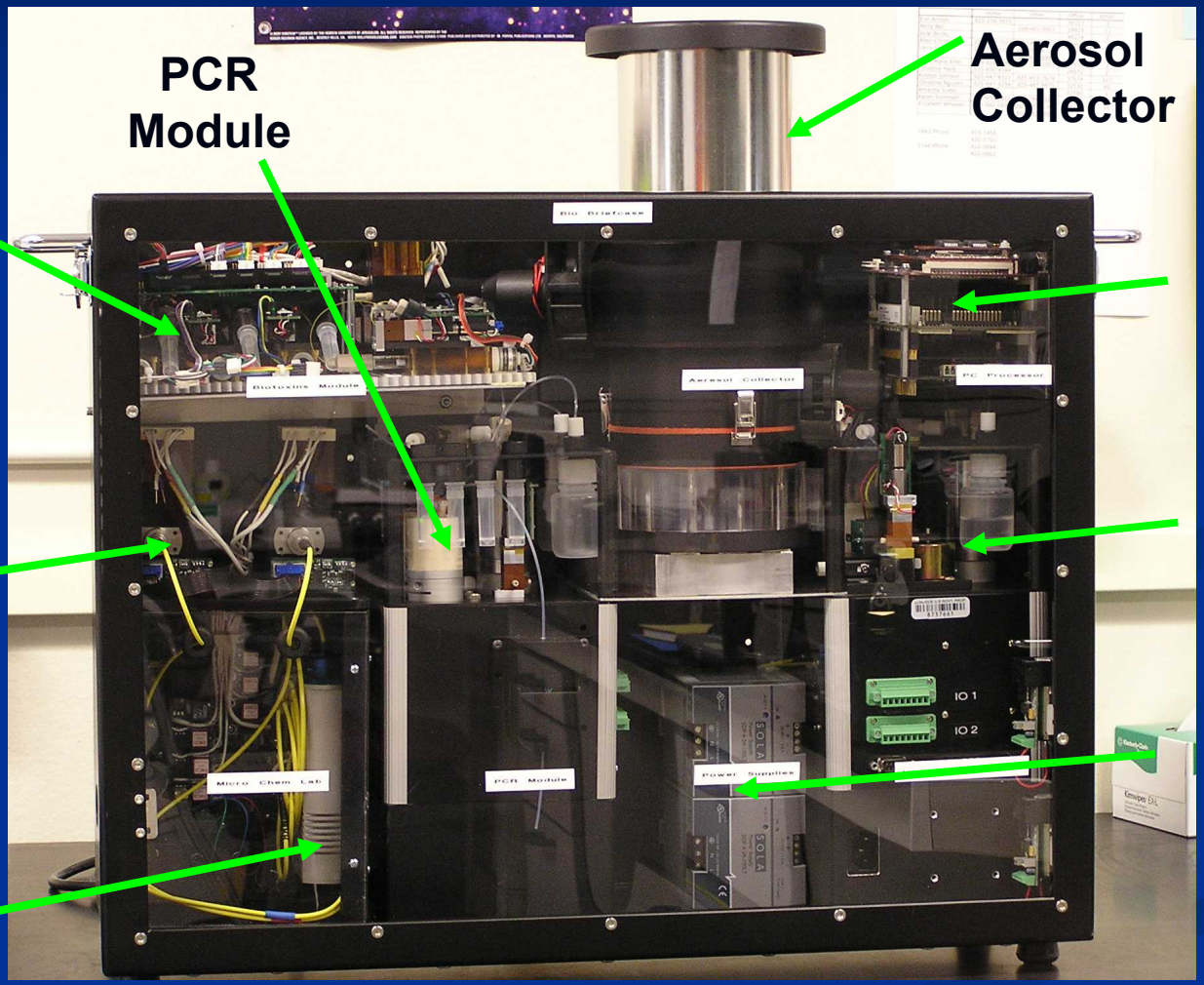
Summary of Test 3 results



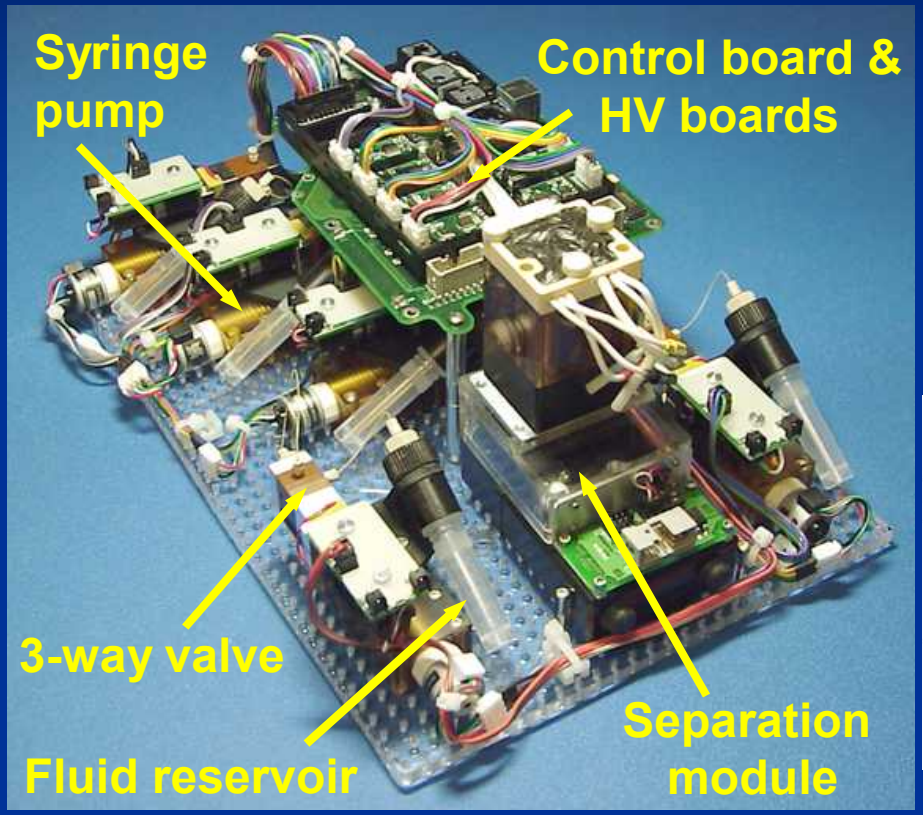
Test results summary

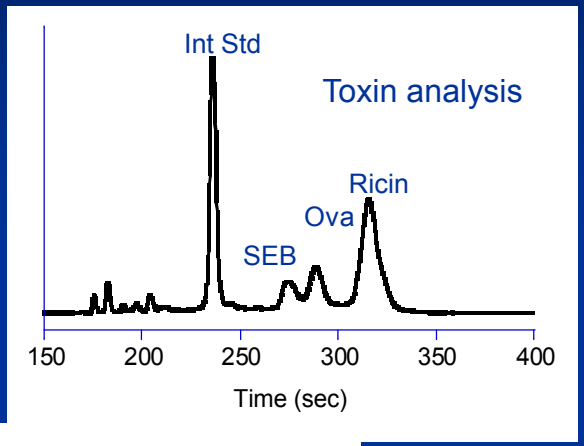
- Tests 3 and 4 are going very well
- Causes of failed detections:
  - Bad PCR reagent lot
    - Address with improved pre-test QC procedures
  - Component failure (e.g., PCR heater)
    - Address with automated diagnostics
  - Temperature effects on CE analyses lead to missed calibration standards
    - Engineered unit will be temperature-controlled
  - Lack of a large training set for analysis software

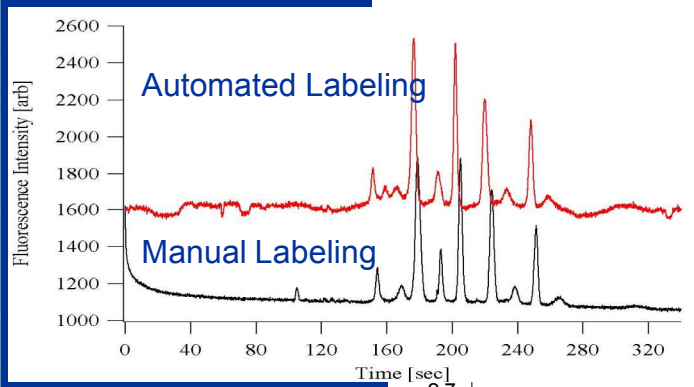
BioBriefcase laboratory components tested at Univ. of Nevada Las Vegas in early 2006

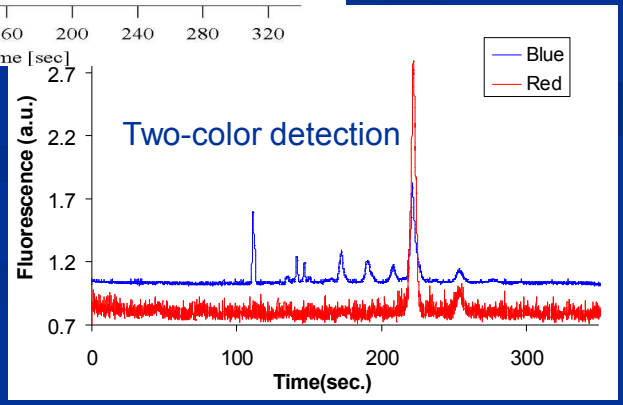


BioBriefcase toxins train directly detects fluorescent dye-labeled toxins using the µChemLab CE/LIF platform









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