



BioBriefcase

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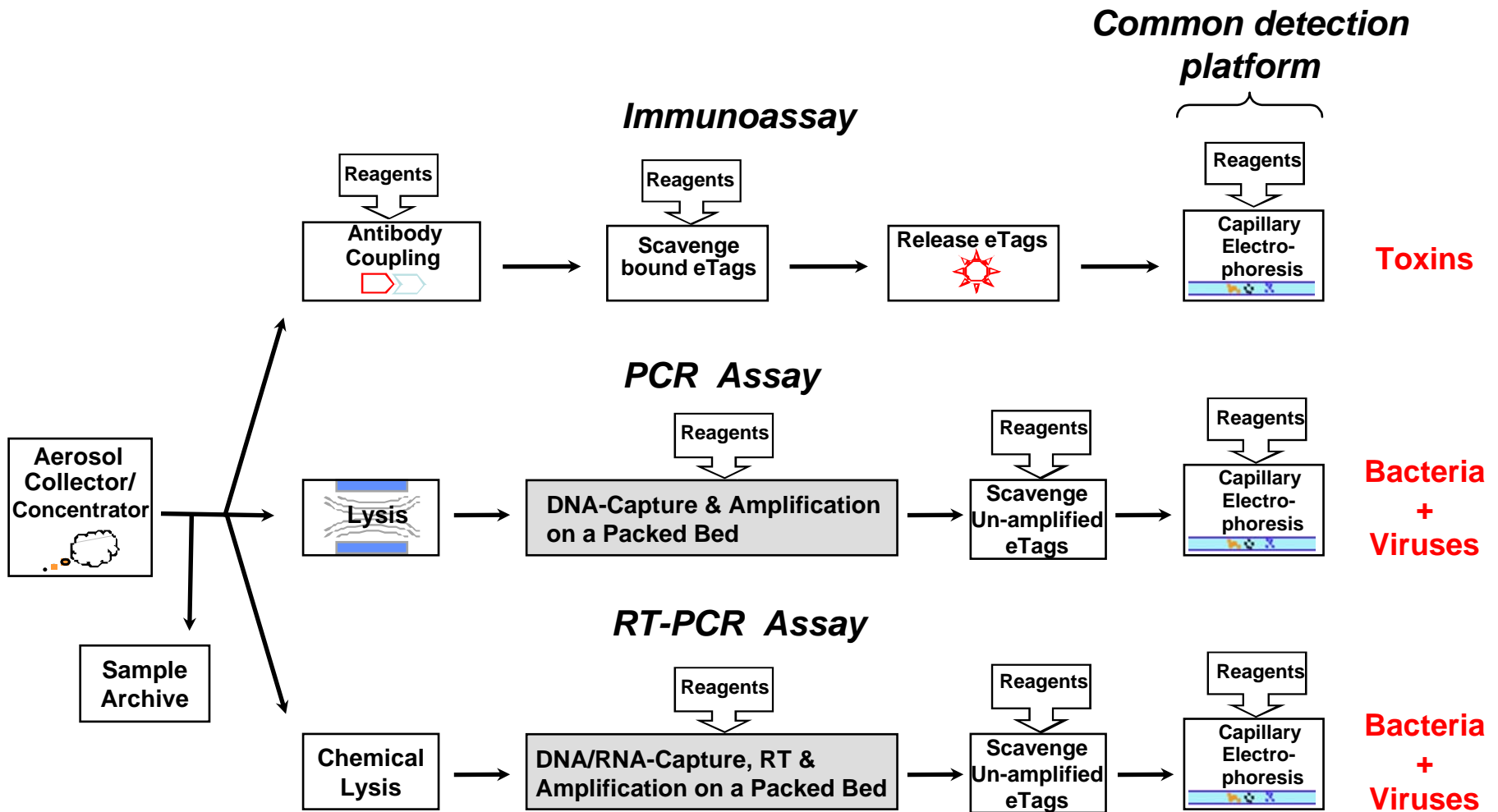


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Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

BioBriefcase employs three analysis trains to detect the full biothreat spectrum (bacteria, viruses and toxins)



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

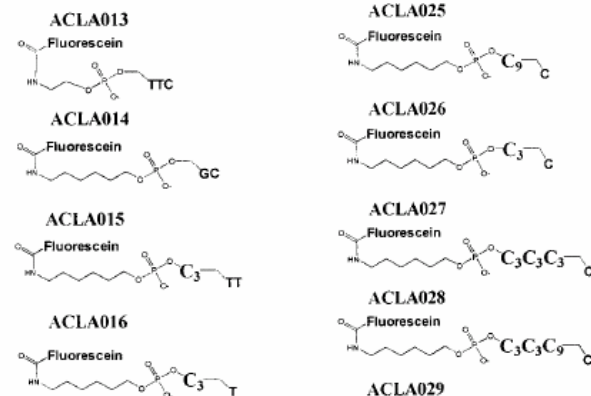
Well characterized electrophoretic mobility

Tagged with same fluorophore

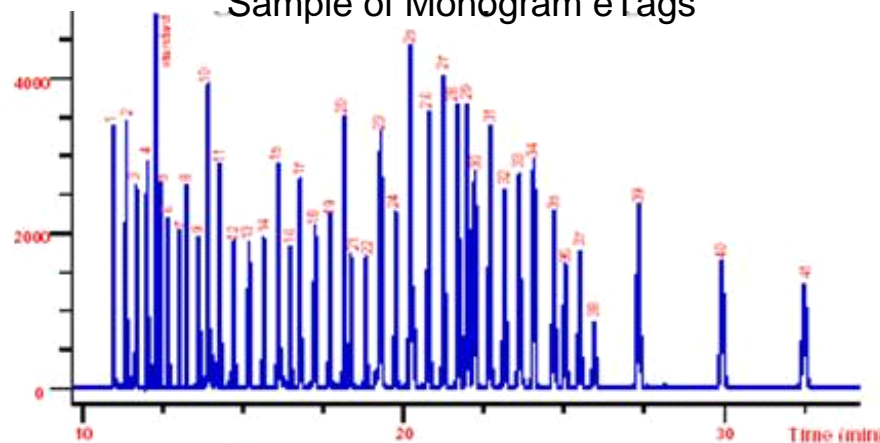
CE Analysis -

Amenable to analysis on chip

Rapid separations



Sample of Monogram eTags

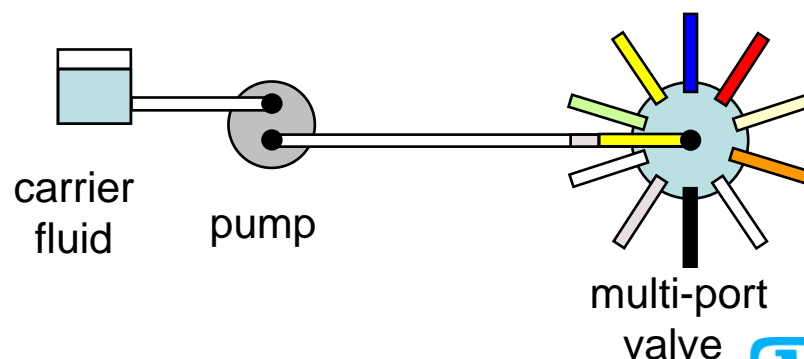


Zone fluidics – automated approach for manipulating fluids for chemical processes

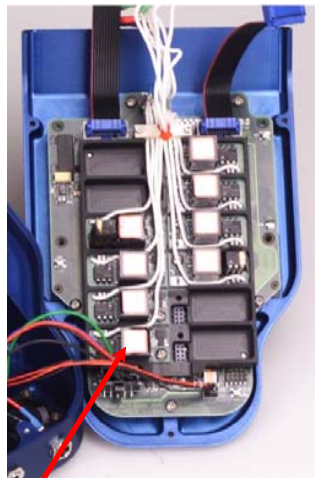
- Can perform most standard unit operations
- Simple components – reversible pump, multi-port selection valve, versatile control software
- Air bubbles are used to separate reagents and minimize diffusion
- Compatible with microfluidics



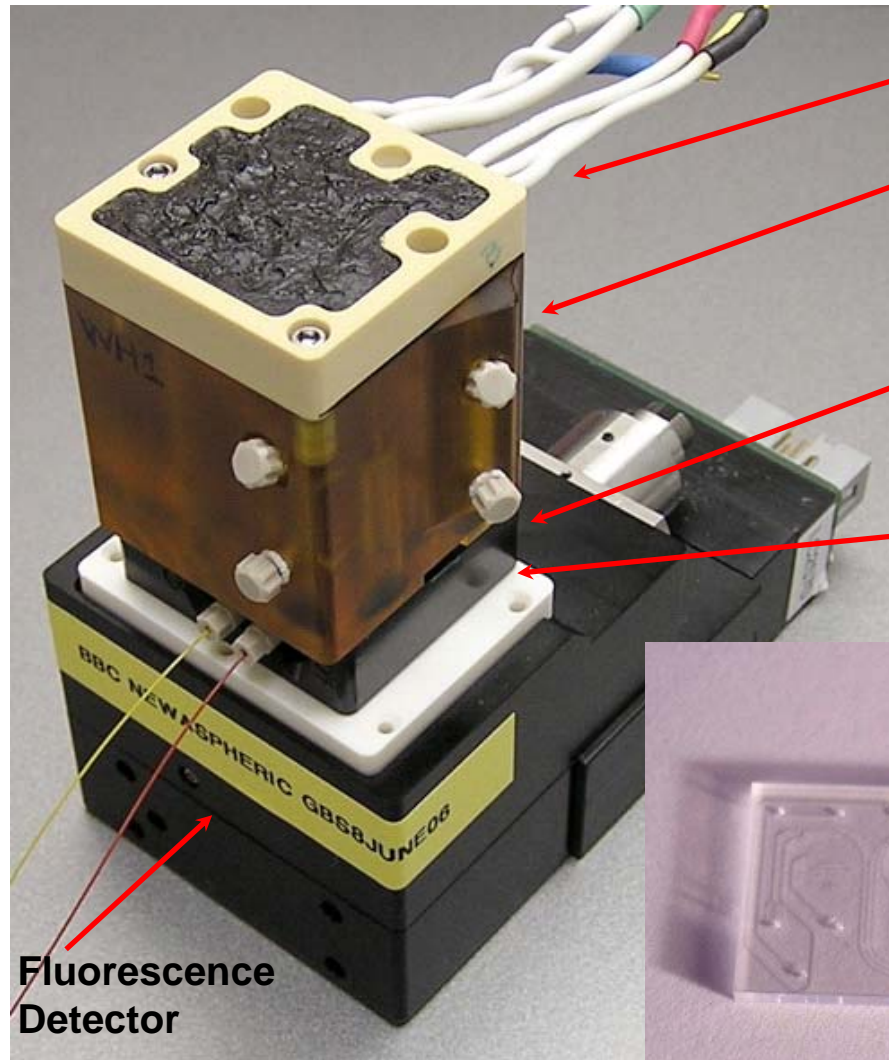
Global FIA mini FloPro



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



High-Voltage
Power Supplies



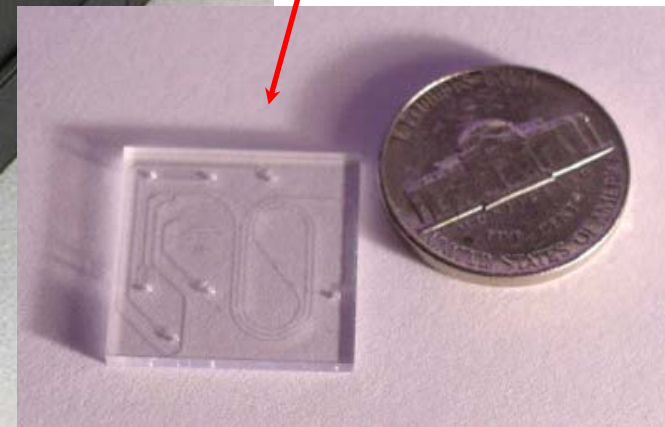
Electrode Plate

Reservoir
Cartridge

Liquid Manifold

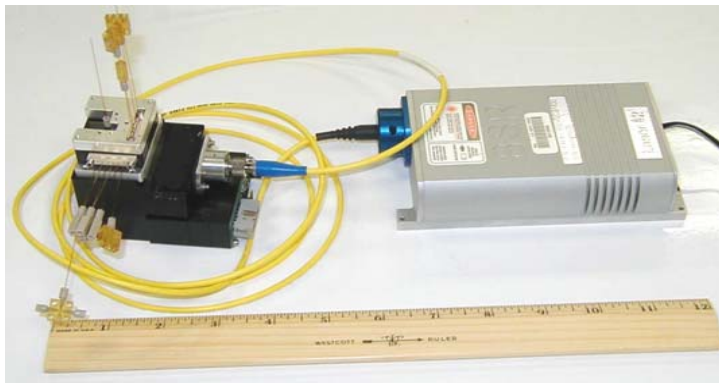
Microchip

Fluorescence
Detector

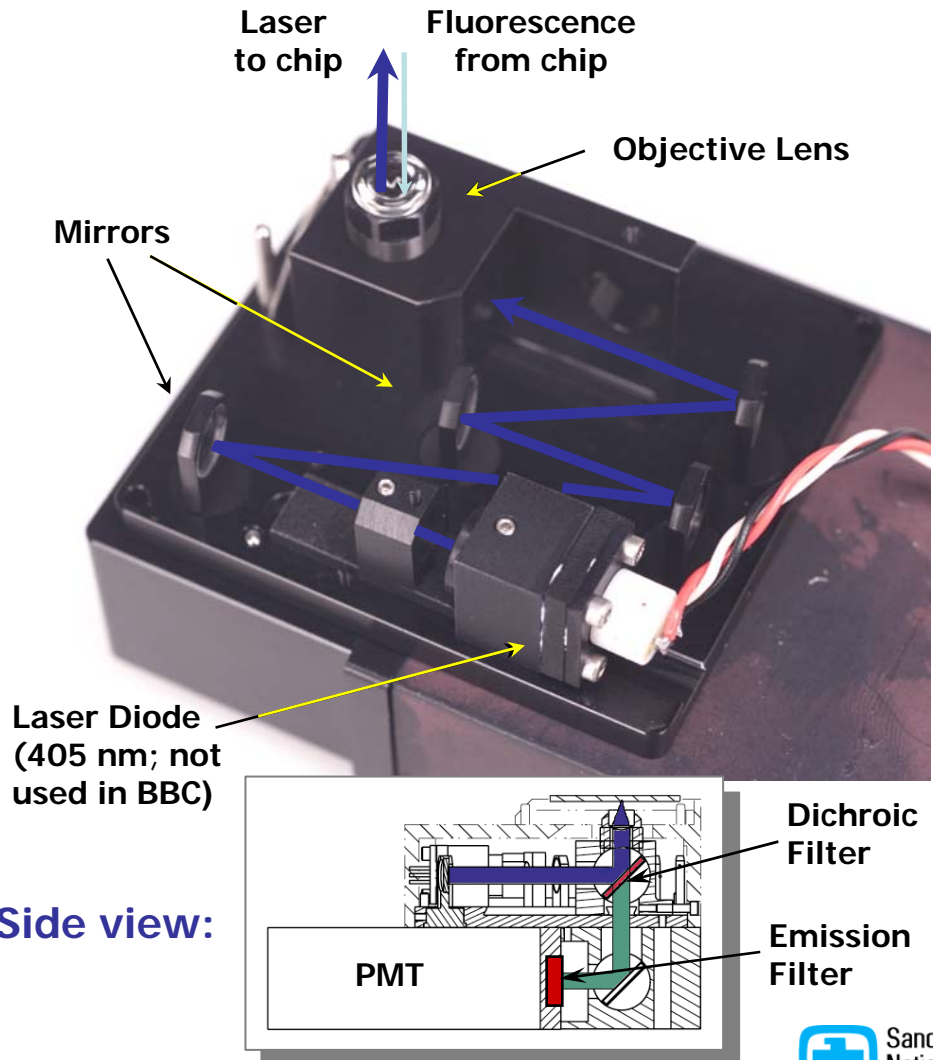


μ ChemLab detector design incorporates sensitive on-chip LIF detection in a compact package

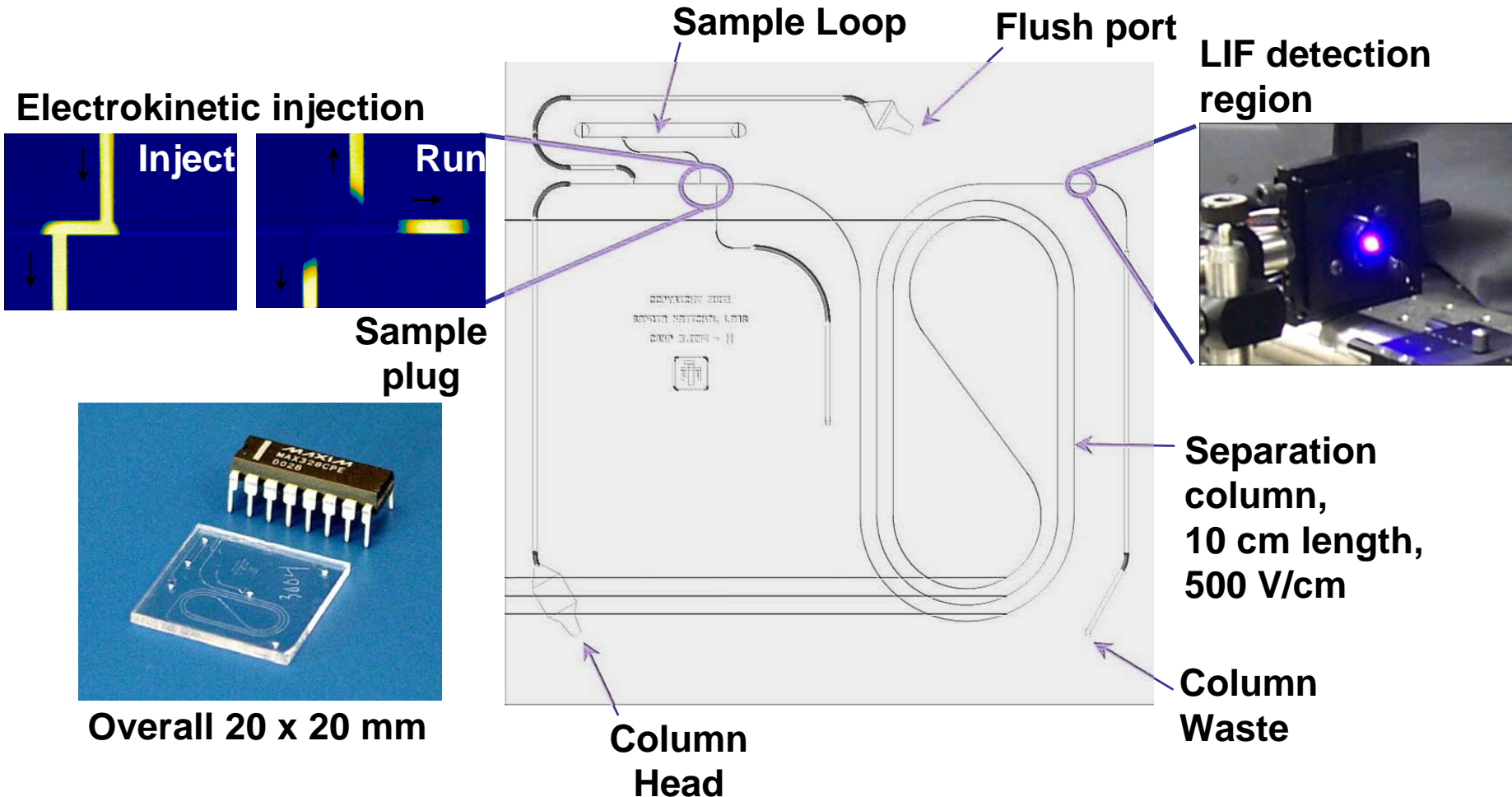
- Epifluorescence configuration
 - High NA, short f.l. objective lens
- Dye-free, single adjustment alignment



Detector fiber-coupled to 488 nm laser detector for eTag assay trains



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

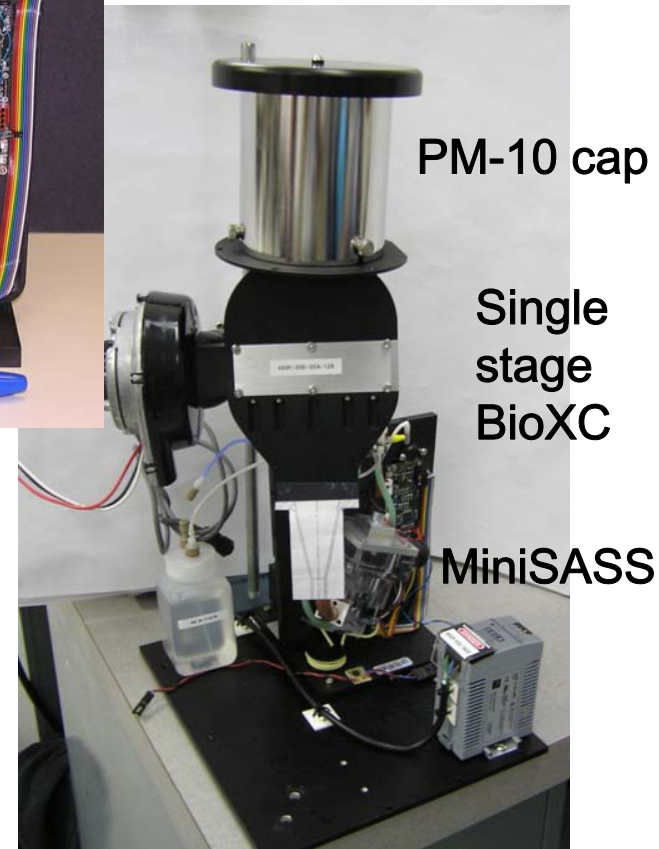


Aerosol collector

- Research International MiniSASS
 - Wetted wall cyclone
 - 30 – 50 Lpm
 - 1 mL sample
 - ~8 L/month water
- MesoSystems BioXC Aerosol Concentrator
 - First stage concentrates from 400 Lpm to 33 Lpm
 - Second stage from 33 Lpm to 3 Lpm
 - We are interested in connecting high flow stage at 450 Lpm flow rate to the MiniSASS at 45 Lpm



MiniSASS

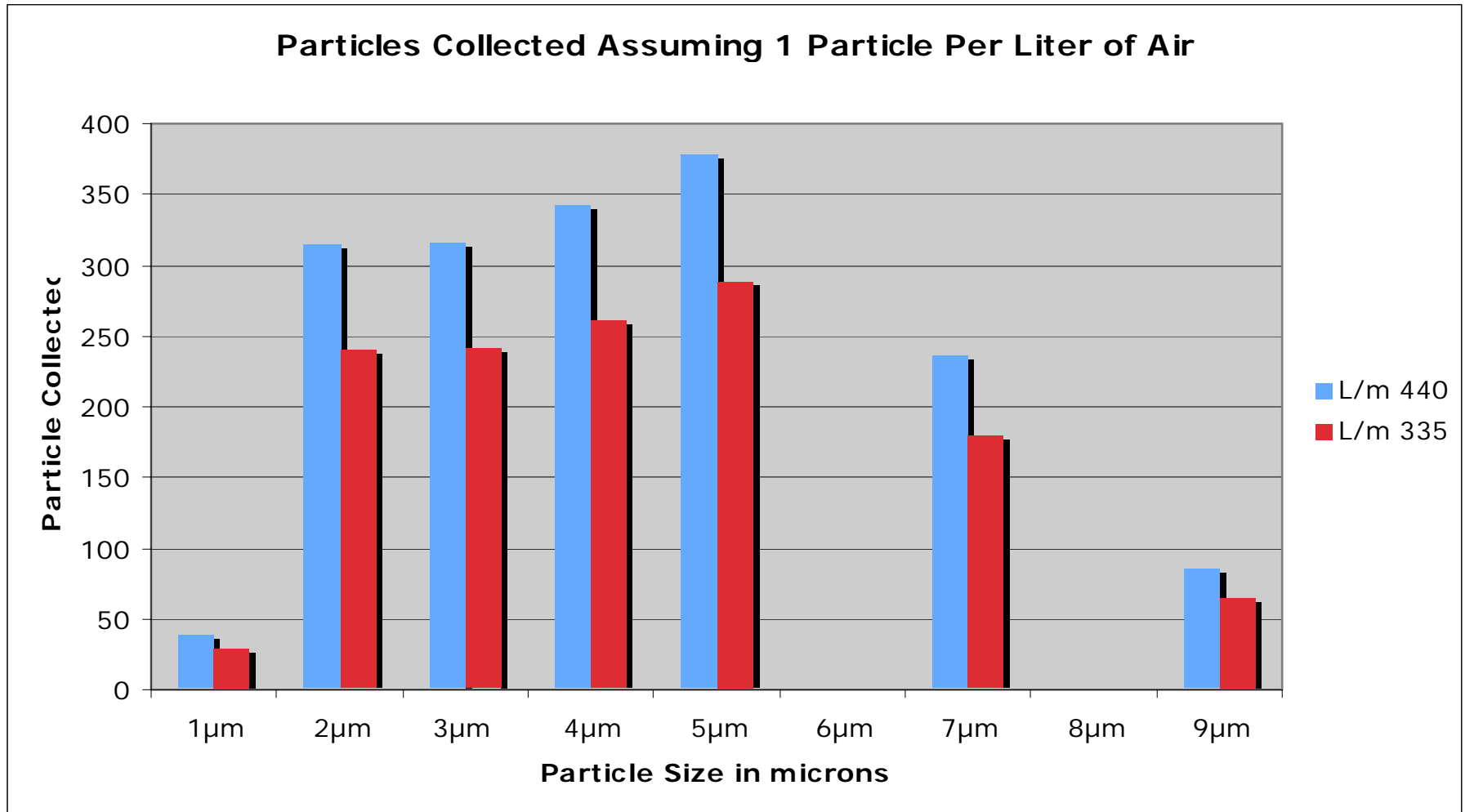


PM-10 cap

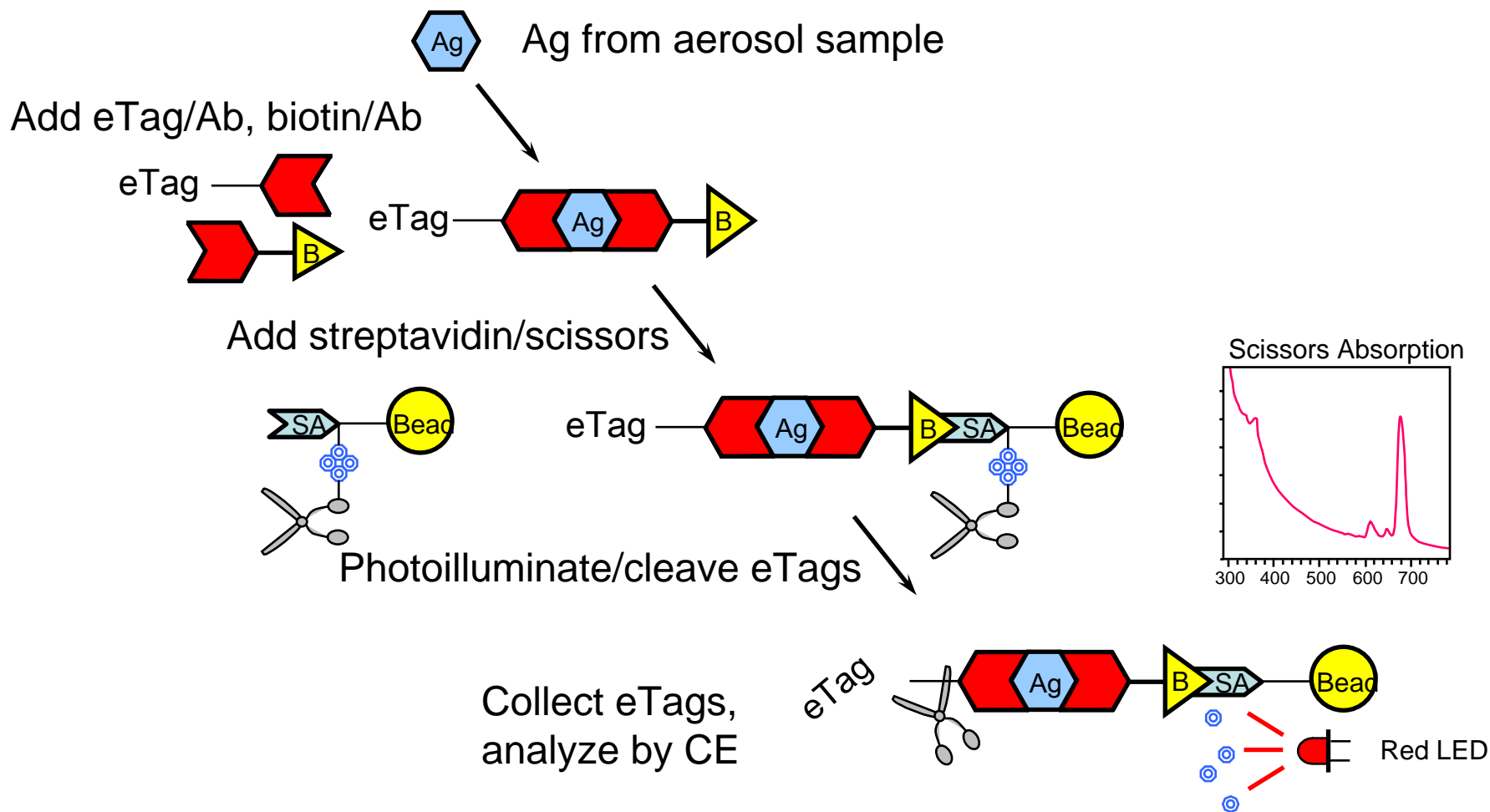
Single stage BioXC

MiniSASS

MesoSystems first stage VI coupled to RI MiniSASS



eTag reporter immunoassay for toxins

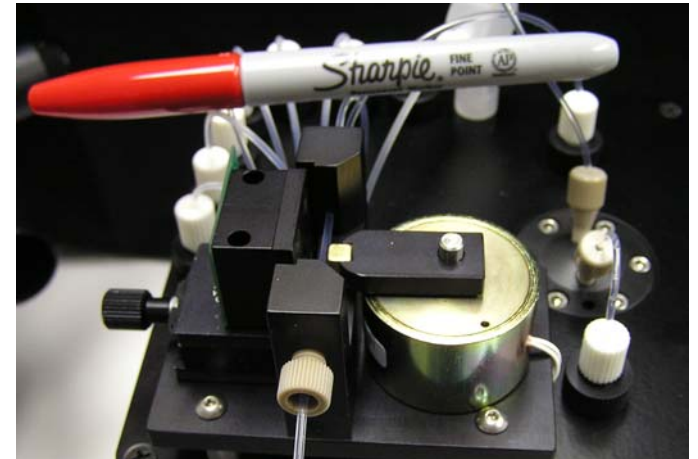


Custom hardware has been developed for flow-through immunoassay

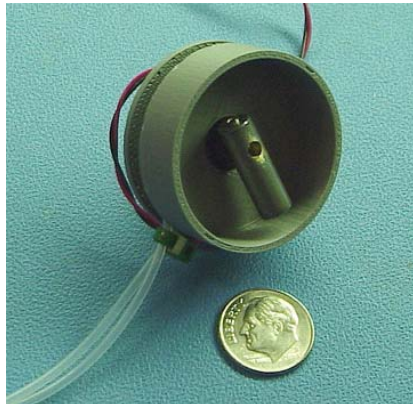
Bead reagent mixer



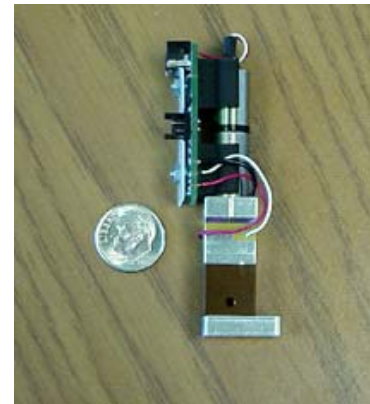
Magnetic trap/
photolysis



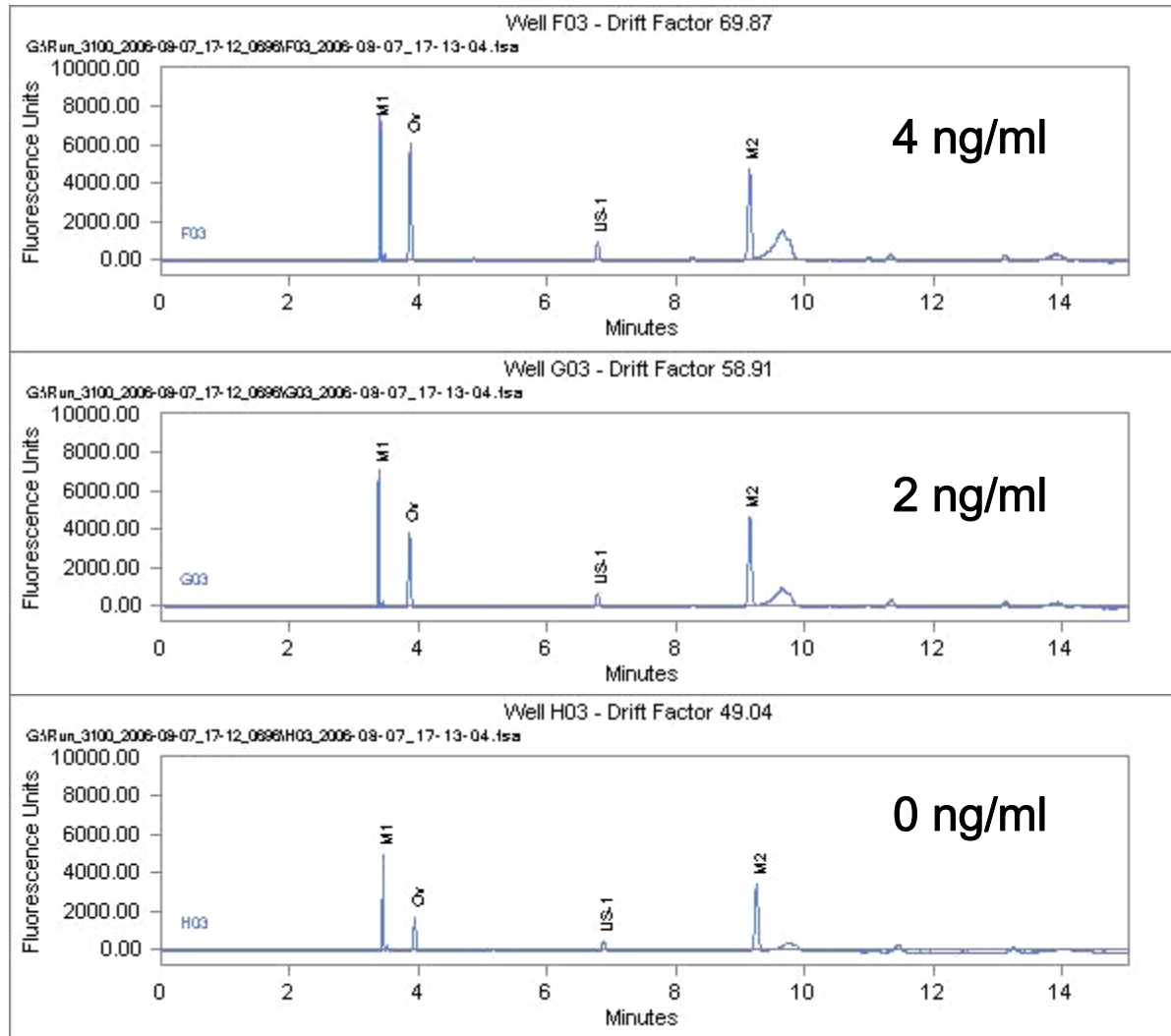
Tube
bead
mixer



3 way valve
< 50 nl dead
volume

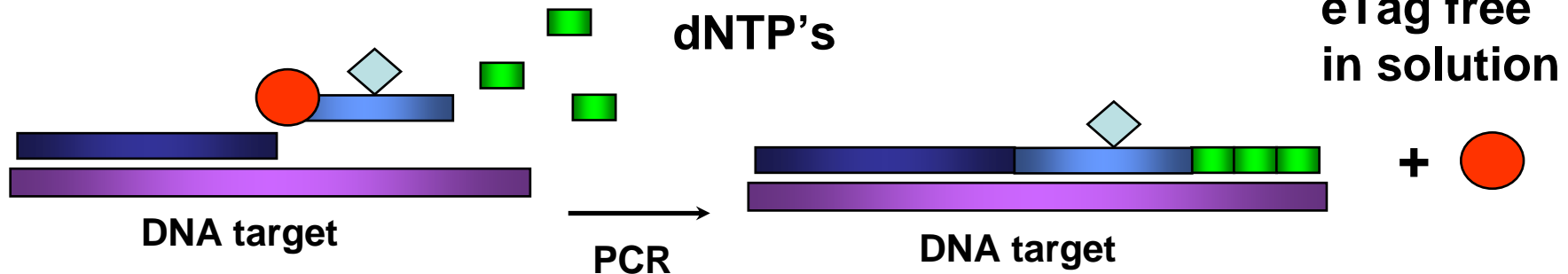


Optimized reactions show excellent ovalbumin sensitivity

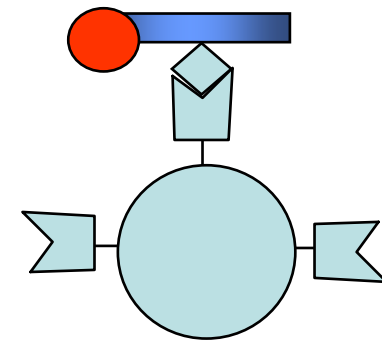


eTag reporter nucleic acid assay for bacteria and viruses

eTag with probe and biotin hook

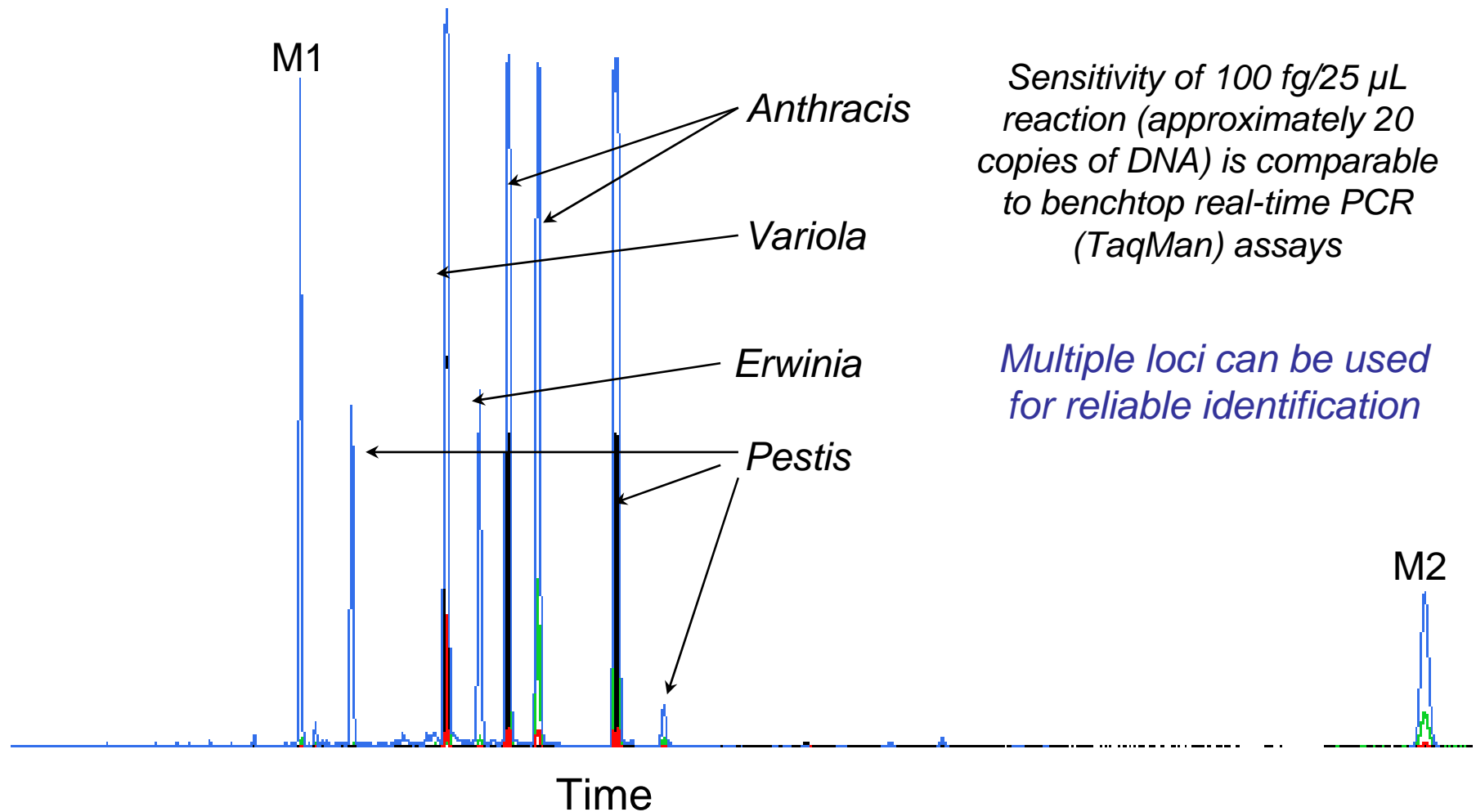


5'-Nuclease activity
of enzyme cleaves
eTag reporters
during PCR
amplification

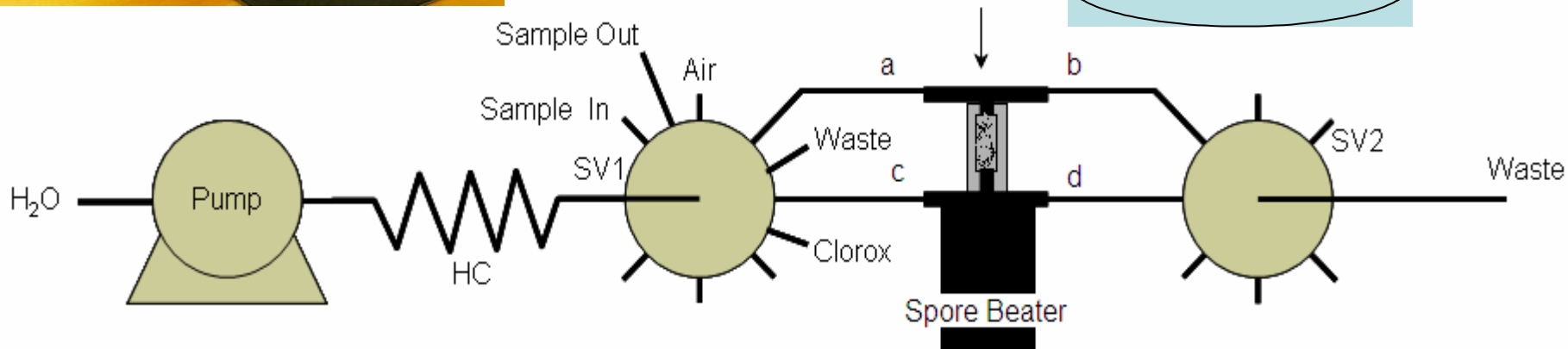
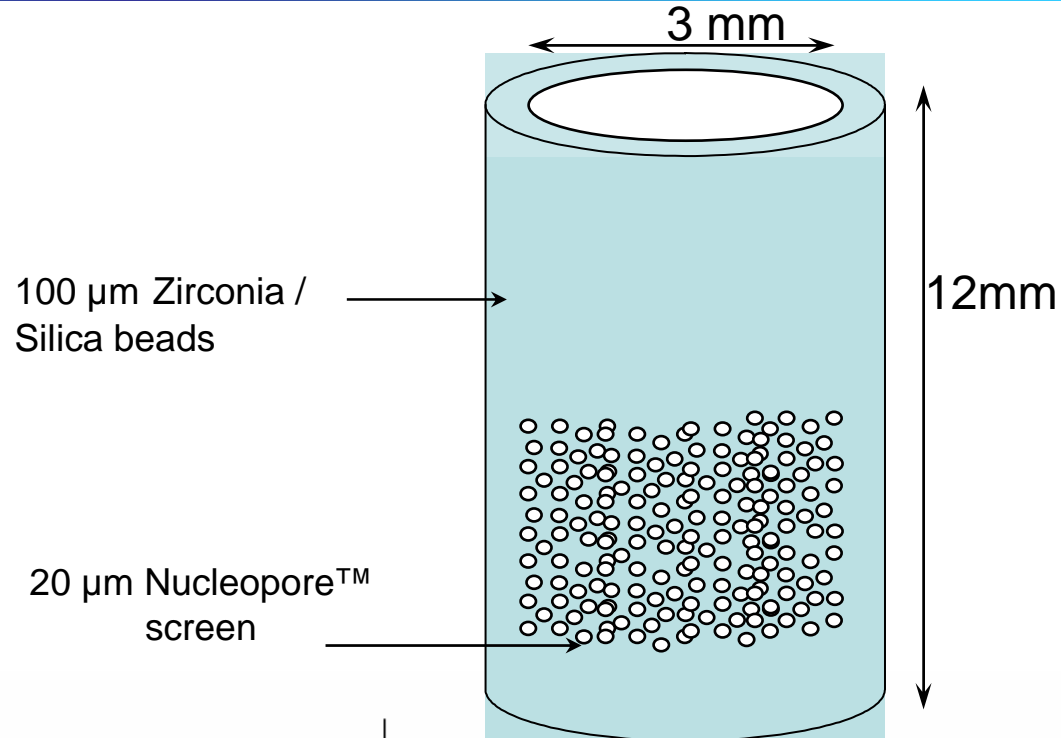


Streptavidin scavenges
free primers

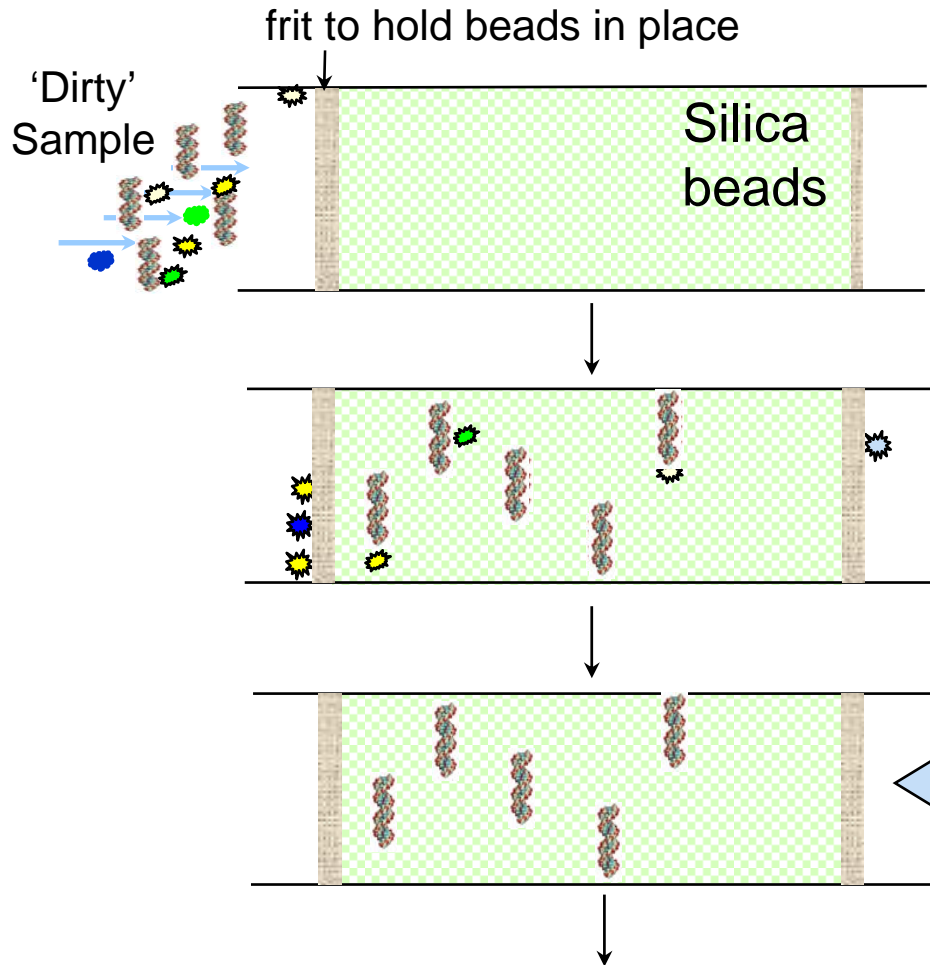
Multiplex nucleic acid eTag assay works in flow-through thermal cycler



Global FIA Spore Beater™ mimics a bead beater in a flow-through configuration



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

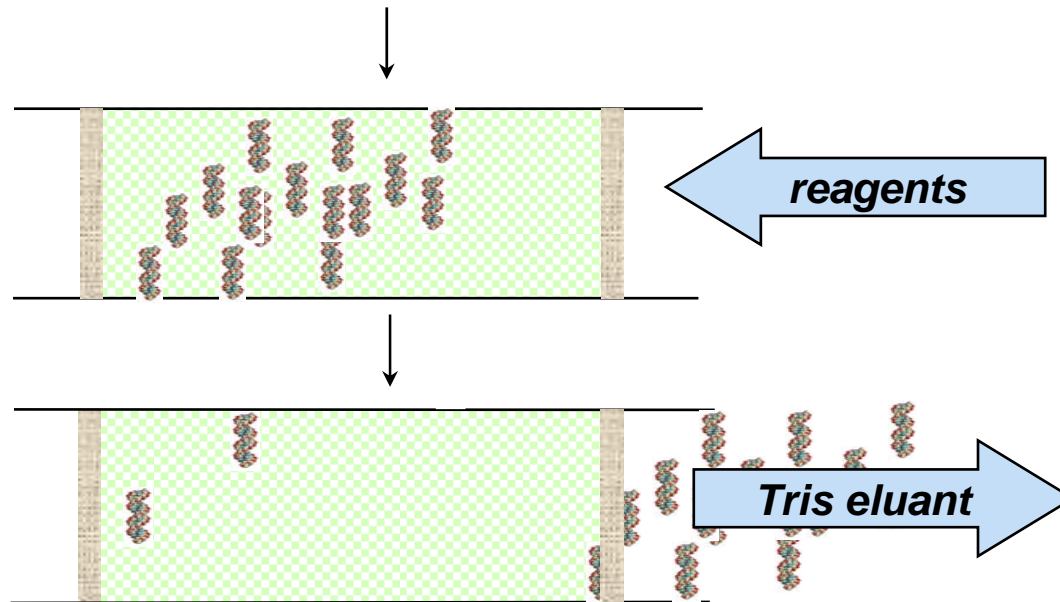
DNA binds to silica beads

wash solutions

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



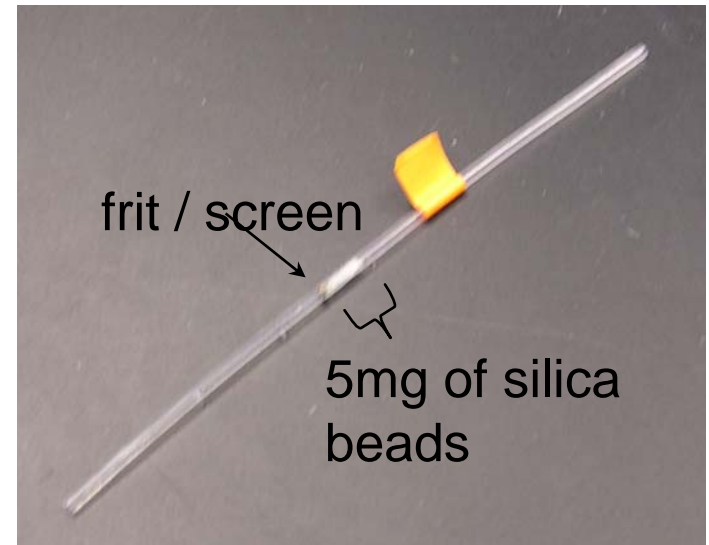
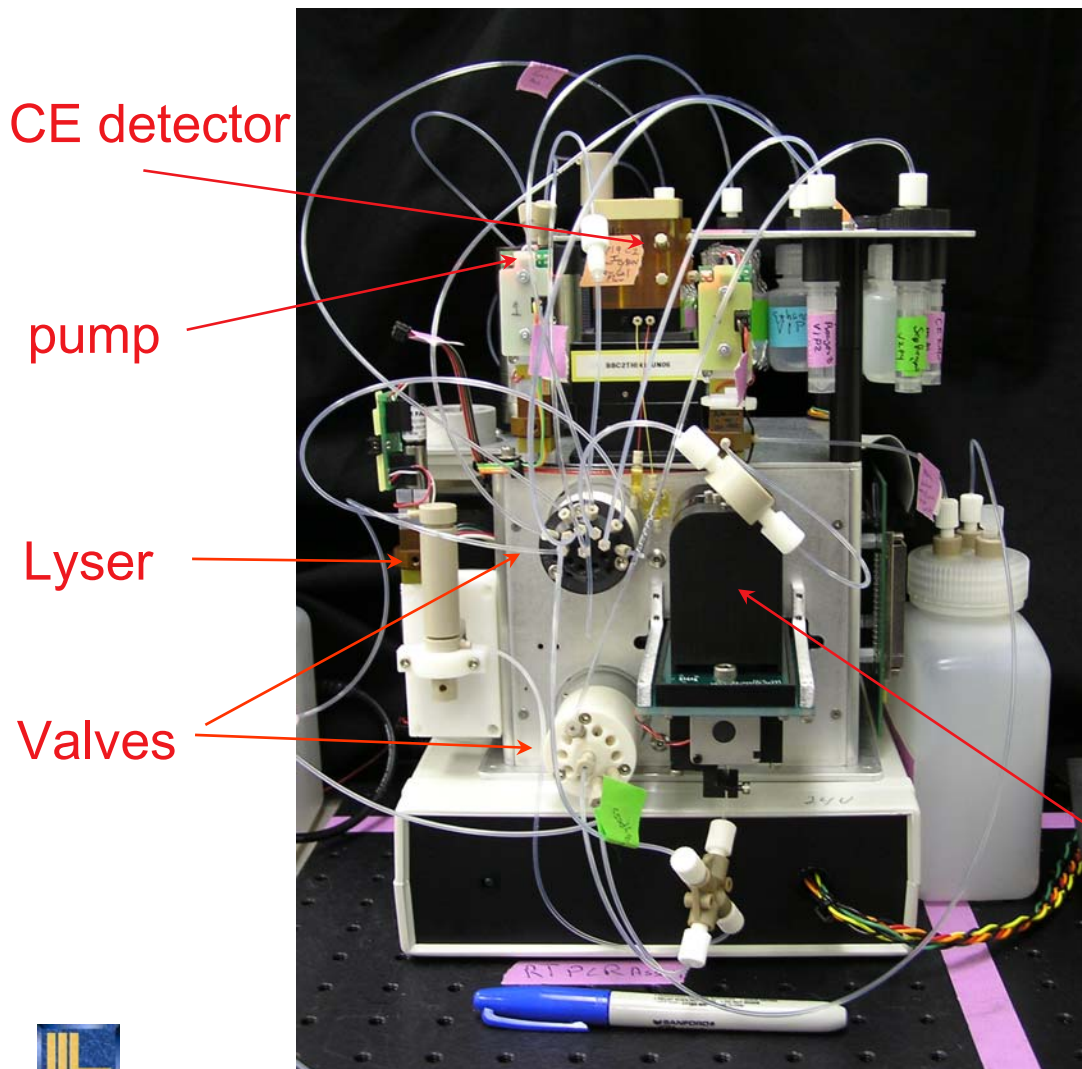
Amplification occurs on bound DNA; DNA is not eluted from surface, reducing losses

Amplified DNA is eluted for analysis

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



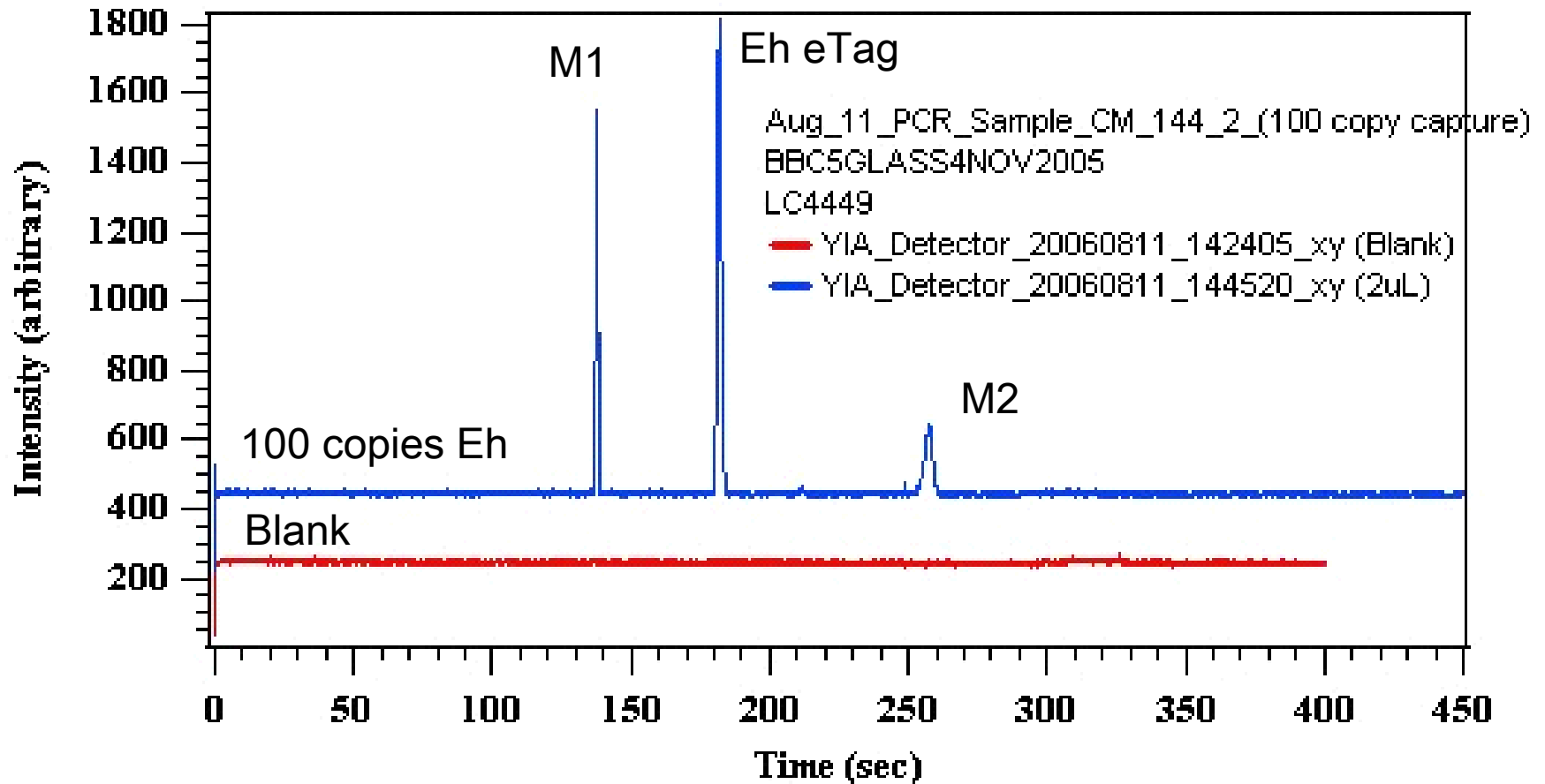
BBC – Lyser, PCR Reactor, CE System



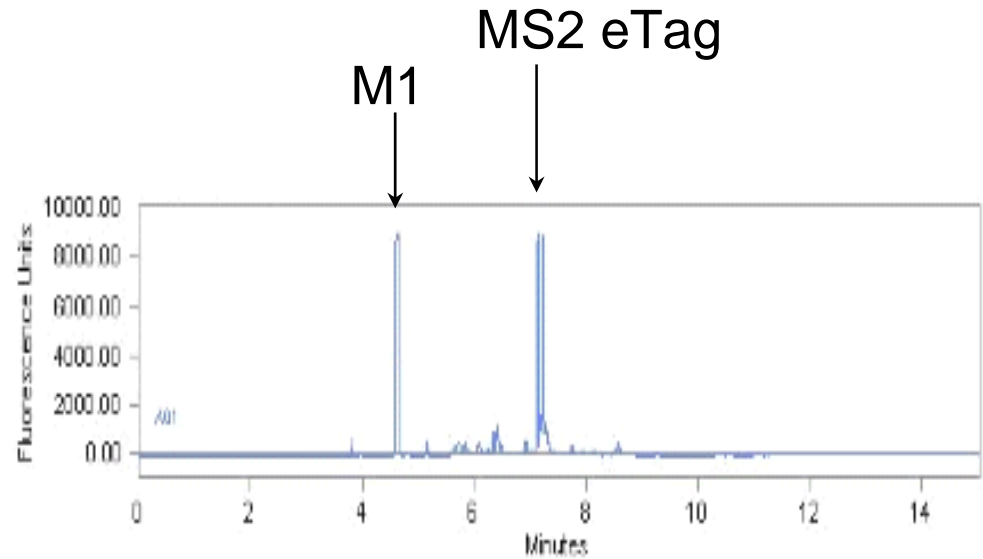
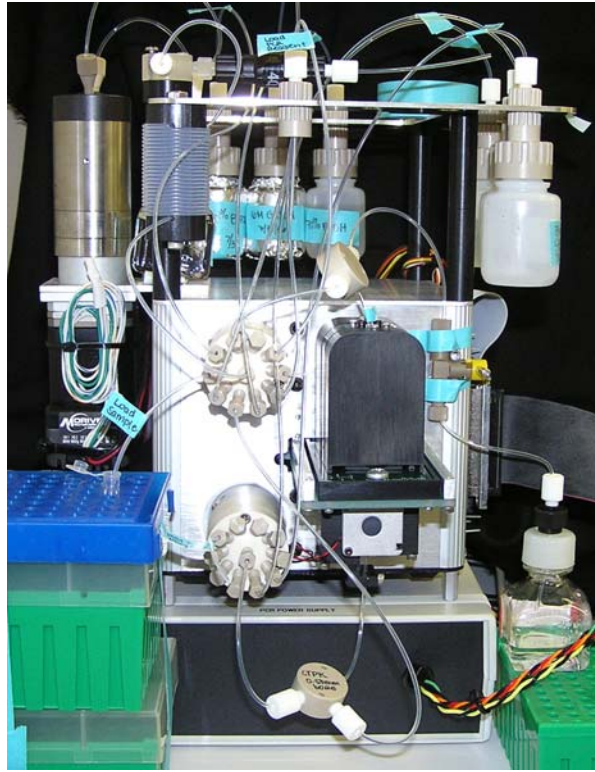
PCR packed bed

PCR chamber

100 copies of *Erwinia herbicola* DNA captured & amplified on automated flow-through platform then analyzed on BBC CE detector



Simulant MS2 successfully amplified on packed bed using autonomous flow-through platform



RT-PCR eTag assay demonstrated on flow-through system

Integrated BioBriefcase containing the three analysis trains, aerosol collector, laser tower and power distribution tower

