Integrated Bioparticle Concentration for Improved Detection in Autonomous Systems

Sample Prep 2011, San Diego, CA

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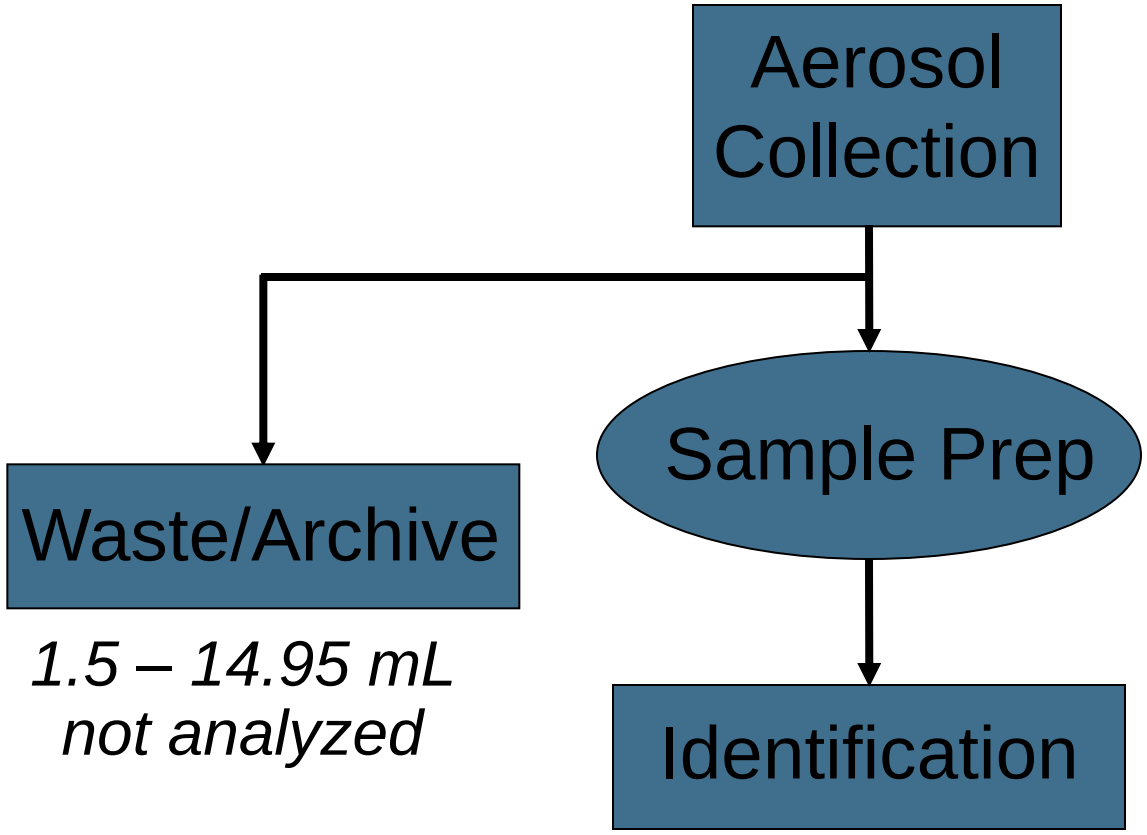
Outline

I. InnovaPrep/Hydrosol Concentration Background
II. Performance
III. Integration with the Evogen Dryclone
IV. Integration for DHS AESaP
V. Integration with MFSI M-BAND

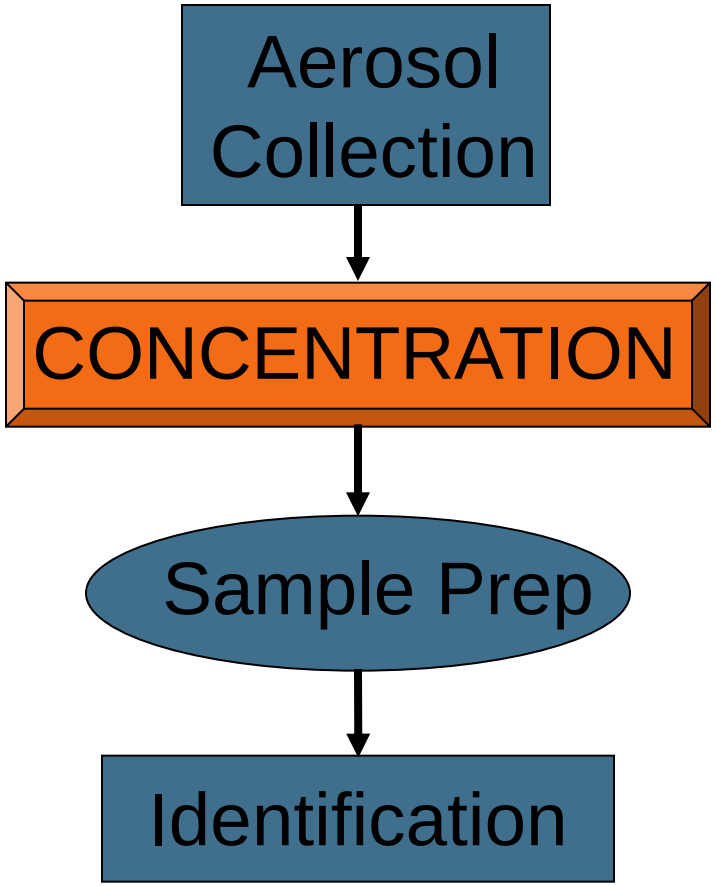
InnovaPrep/Hydrosol Concentration Background

Automated Detection of Aerosolized Biological Weapons – Standard Practice



*75% to more than 99% of the sample is not analyzed!

Automated Detection of Aerosolized Biological Weapons – With Concentration



Sample collection into 2 – 15 mL

Interface between collected sample volume and accepted sample prep volume – "Macro-to-Micro Interface"

Sample prep modules generally accept 50 – 500 µL

Identifiers accept 5 - 200 µL

*Delivery of nearly 100% of the collected signature is possible – providing orders of magnitude improvement in DL

INNOVAPREP

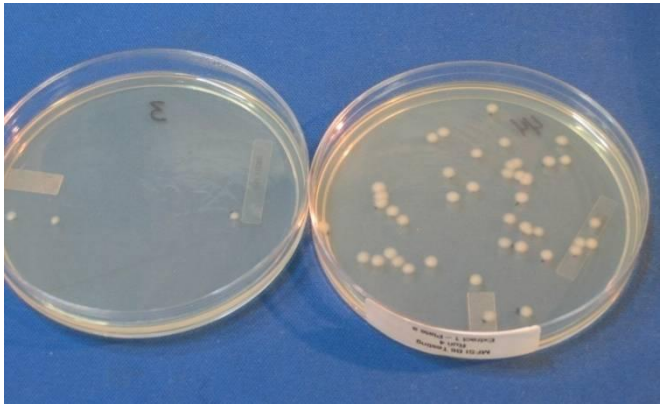
Implementation of a Hydrosol Concentrator

Advantages

- Orders of magnitude improvement in detection limit
- Increased freedom in selection of collector
- Increased freedom in selection of detector

Disadvantages

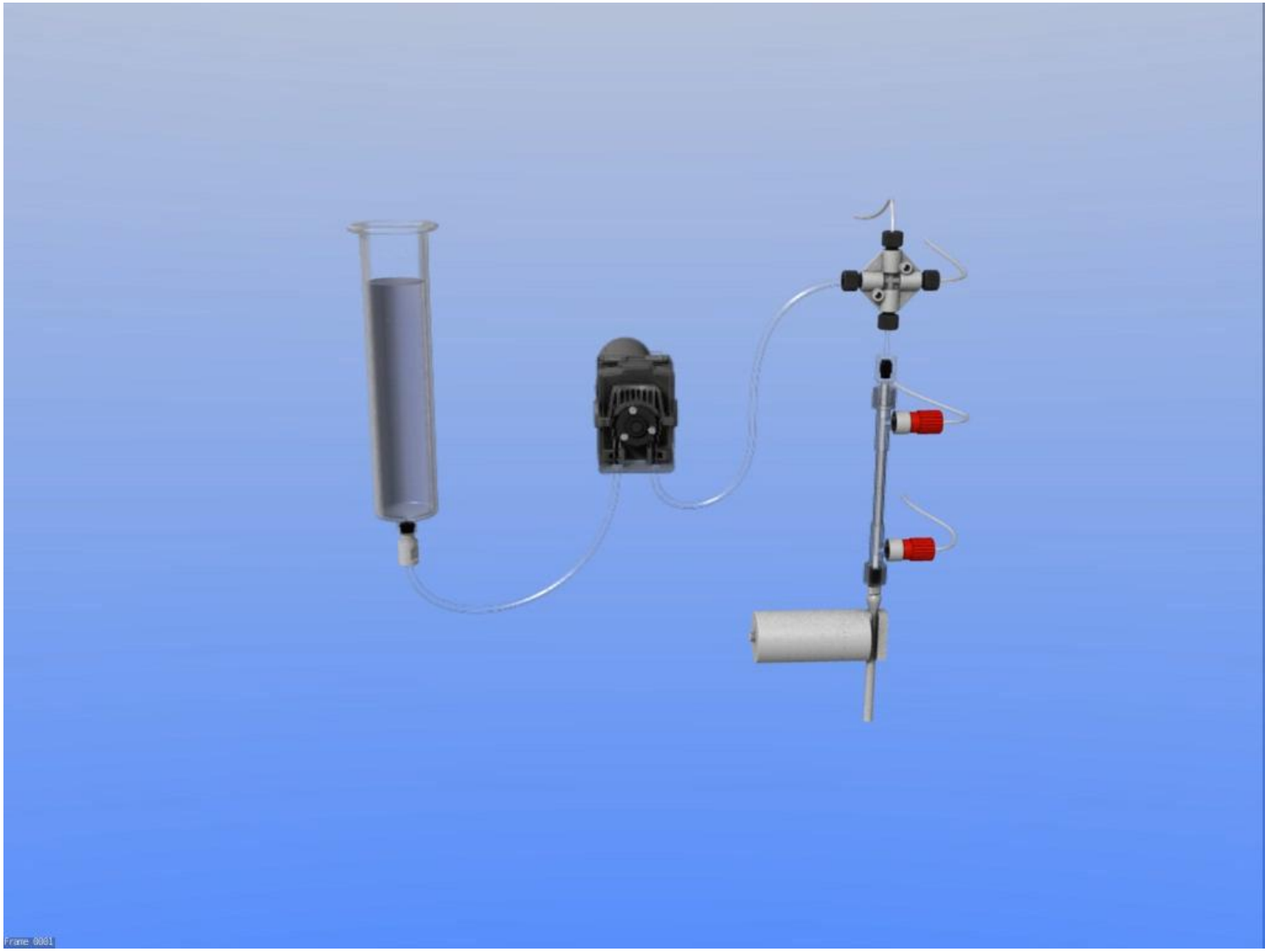
- It adds another component to the system
- It takes additional time
- Co-concentration of Inhibitors is possible



The InnovaPrep Concentration Process

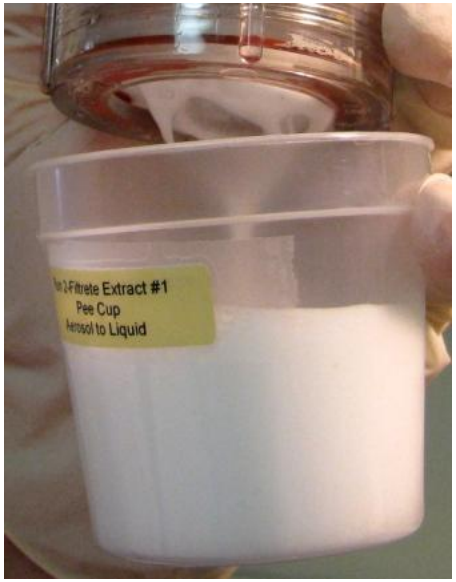
- “Dead-end” filtration through a hollow fiber or flat membrane filter
- Particles larger than the filter pore size are retained while the permeate fluid passes through
- A viscous, expanded wet foam is pushed through the retentate tangential to the filter surface capturing the particles

The InnovaPrep Concentration Process



Wet Foam Elution

- Mode of Operation
  - "Expanded Liquid"
  - Increased Viscosity – reduced channeling (Yan et al., 2006)
  - Moves as rigid body w/ narrow (<10 µm) lubricating layer (Briceno and Joseph, 2003; Tisne et al., 2004)
  - Bursting bubbles
- Improved Elution
  - Lower minimum elution volumes
  - Higher elution efficiencies
  - Largely unaffected by sample matrix
  - Dramatic advantage for multi-fiber & flat membrane modules
- Quickly Breaks Down into a Liquid



InnovaPrep Biological Concentrators

HCI-40 (patents pending)

HSC-40 (patents pending)

Performance

Performance Metrics
(for Autonomous Biothreat Detection Systems)

- Speed
  - Generally less than 5 to 10 minutes is desired
  - Often less than 1 or 2 minutes is desired
- Reusability
  - Reuse for hundreds of test runs is desired
  - Must withstand inhibitor materials (blinding)
- Efficiency/Concentration Factor
  - >50% efficiency is desired
  - >5x concentration factor desired
- Low instrument cost
- Low per sample cost

Speed

Speed is tied to:

- Membrane pore size
- Membrane surface area
- Sample matrix/particle loading & makeup

*For most biodefense applications bacteria, viruses, free DNA, proteins and toxins are concentrated into a single fraction. Approximates rates are:

~1 mL/min to concentrate into <200 uL

~3 mL/min to concentrate into <400 uL

Reusability

Reusability is primarily tied to the parameters listed below

- Membrane surface area
- Sample matrix/particle loading and makeup

*Reuse for up to 250 test runs has been demonstrated

Efficiency

Concentration and Recovery of Carboxylate Polystyrene Microspheres

Particle Size, µm	Represented Particle	Average Recovery
4.5	Agglomerates of bacteria	97%
1.0	Single bacteria	90%
0.05	Viruses	79%
0.025	DNA, Lower limit-viruses	60%

Efficiency

Concentration and Recovery of Microorganisms

Species	Concentration efficiency
Bacillus thuringiensis israliensis (Bti)	97 %
Bacillus atrophaeus (Bg)	95%
Escherichia coli (Ec)	103%
MS-2 bacteriophage	92%
Bg DNA	60%

Cost

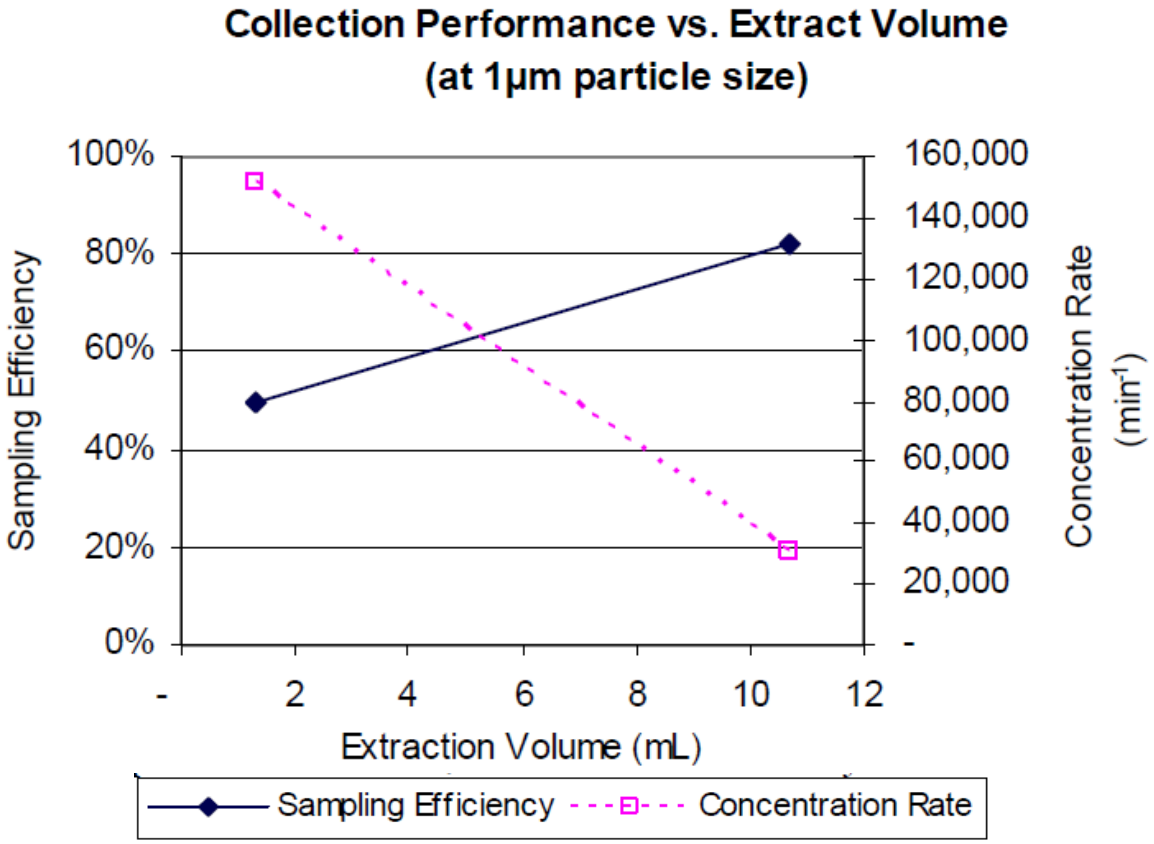
- Instrument is a relatively simple, compact electro-mechanical instrument and thus cost is relatively low.
- Sharing of common components can reduce cost further.
- Per sample cost is very low due to reuse of concentration cells.

Integration with the Evogen DryClone

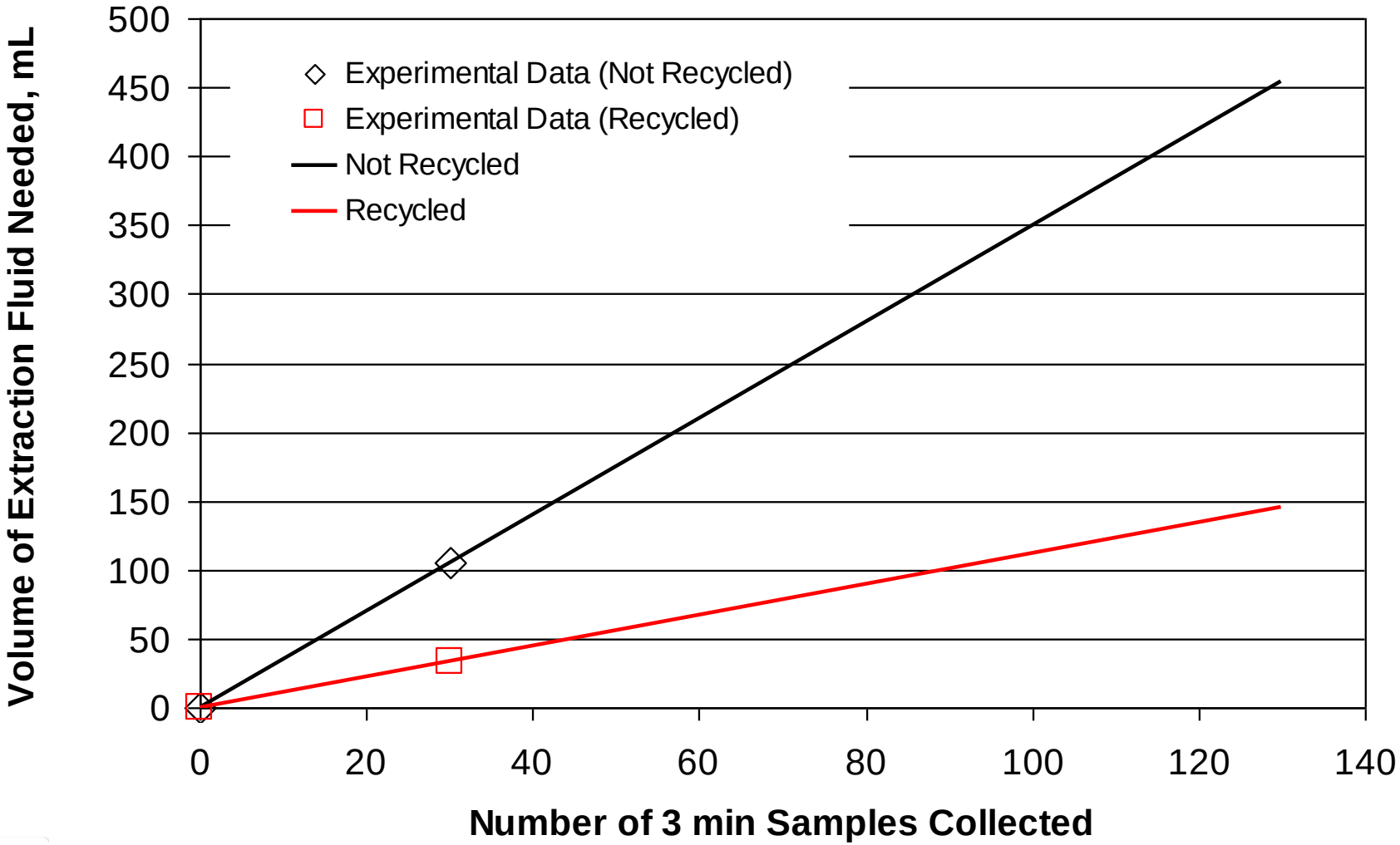
Integration for Concentration and Fluid Reuse

Integration with the Evogen DryClone

- Cyclonic Collector w/ automated wet rinse
- Concentrate Volume Range Tested - 175 to 250 µL
- Demonstrated Concentration Factor - 3.1 to 20.1
- Demonstrated Concentration Efficiency – 78% to 115%
- Fluid Reuse Demonstrated



Fluid Reuse


Volume of Extraction Fluid Needed, mL
500
450
400
350
300
250
200
150
100
50
0
Experimental Data (Not Recycled)
Experimental Data (Recycled)
Not Recycled
Recycled
0
20
40
60
80
100
120
140
Number of 3 min Samples Collected

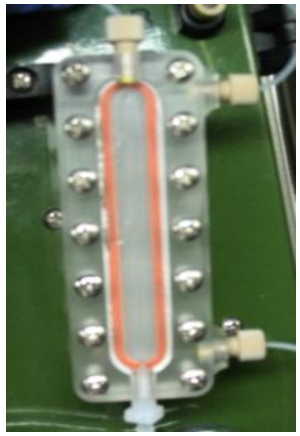
Integration for DHS AESaP

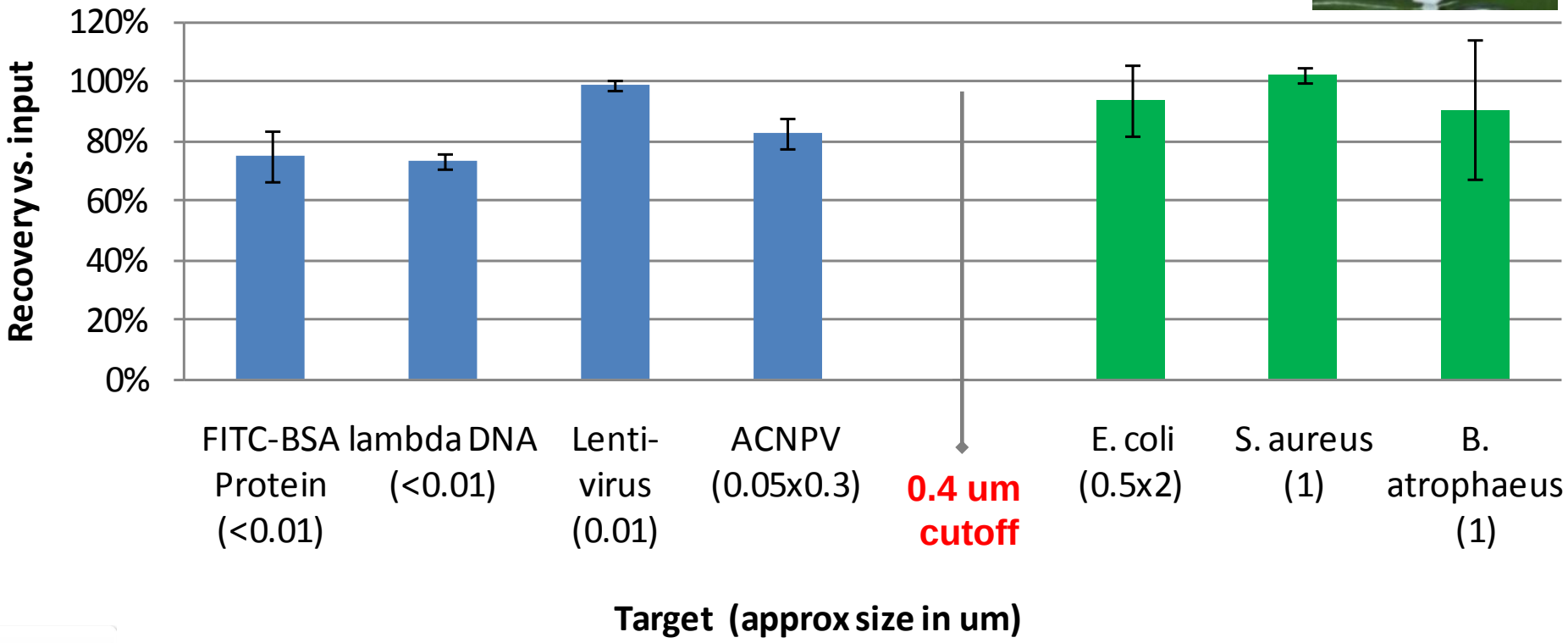
US Department of Homeland Security

Automated Environmental Sample Preparation Program (AESaP)

Passage & Concentration Recoveries

- Flat membrane
- Efficiency for passage of viruses, proteins, DNA
- Efficiency for concentration of bacteria



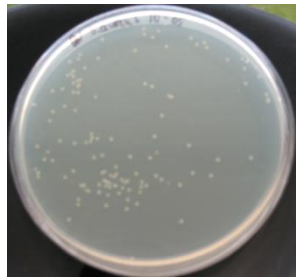


*Data provided by Darren Gray, FLIR

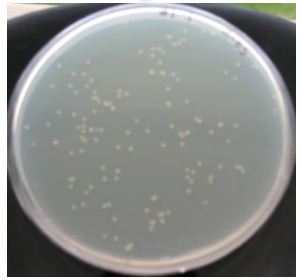
High Yield of Viable Vegetative Bacteria

- Flat membrane
- Yield by qPCR
- Viability by plating

	Innovaprep Yield	Viability (relative to input)	Process yield of viable cells
E. coli	94 ± 15%	77 ± 23%	**71 ± 16%**
S. aureus	102 ± 3%	98 ± 3%	**100 ± 6%**



InnovaPrep input



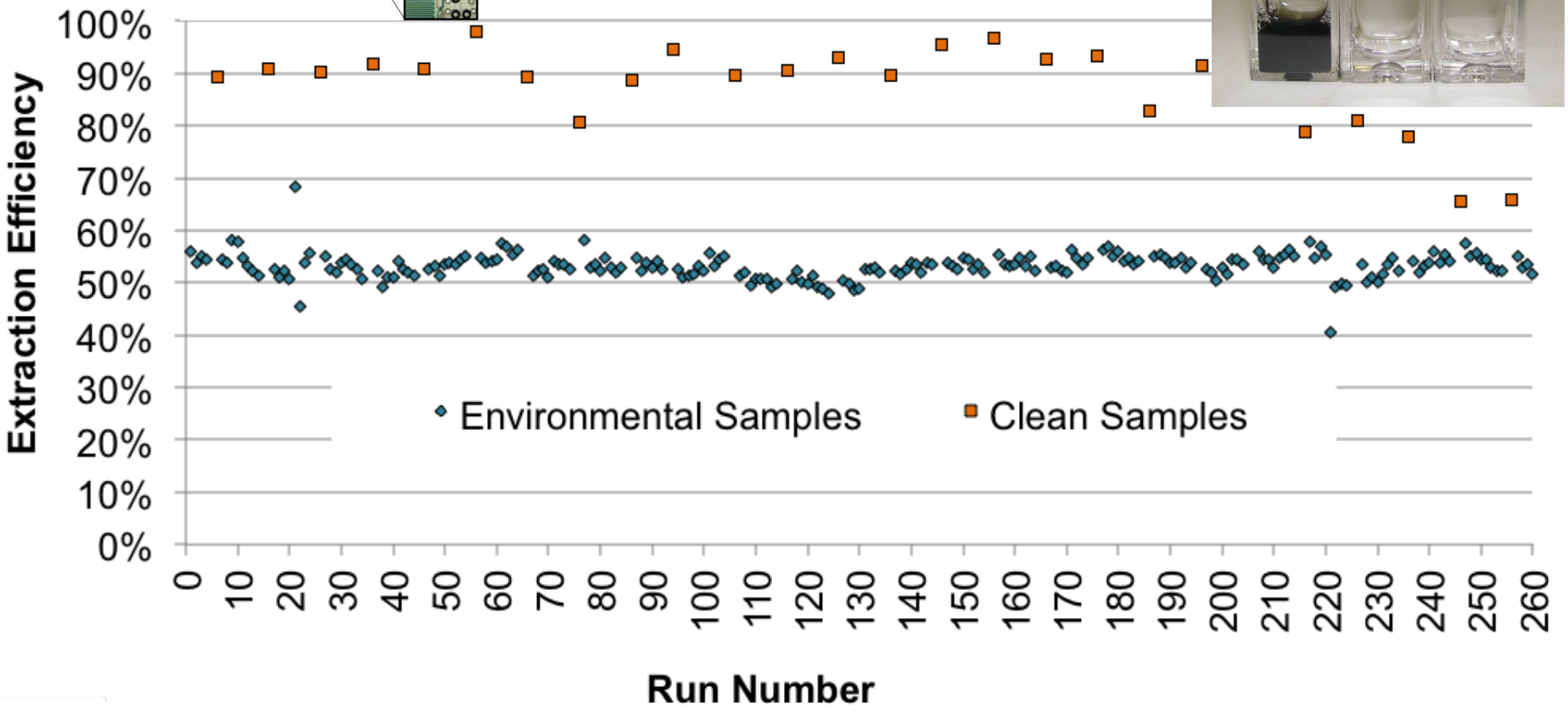
Cell Fraction output

**Data provided by Darren Gray, FLIR*

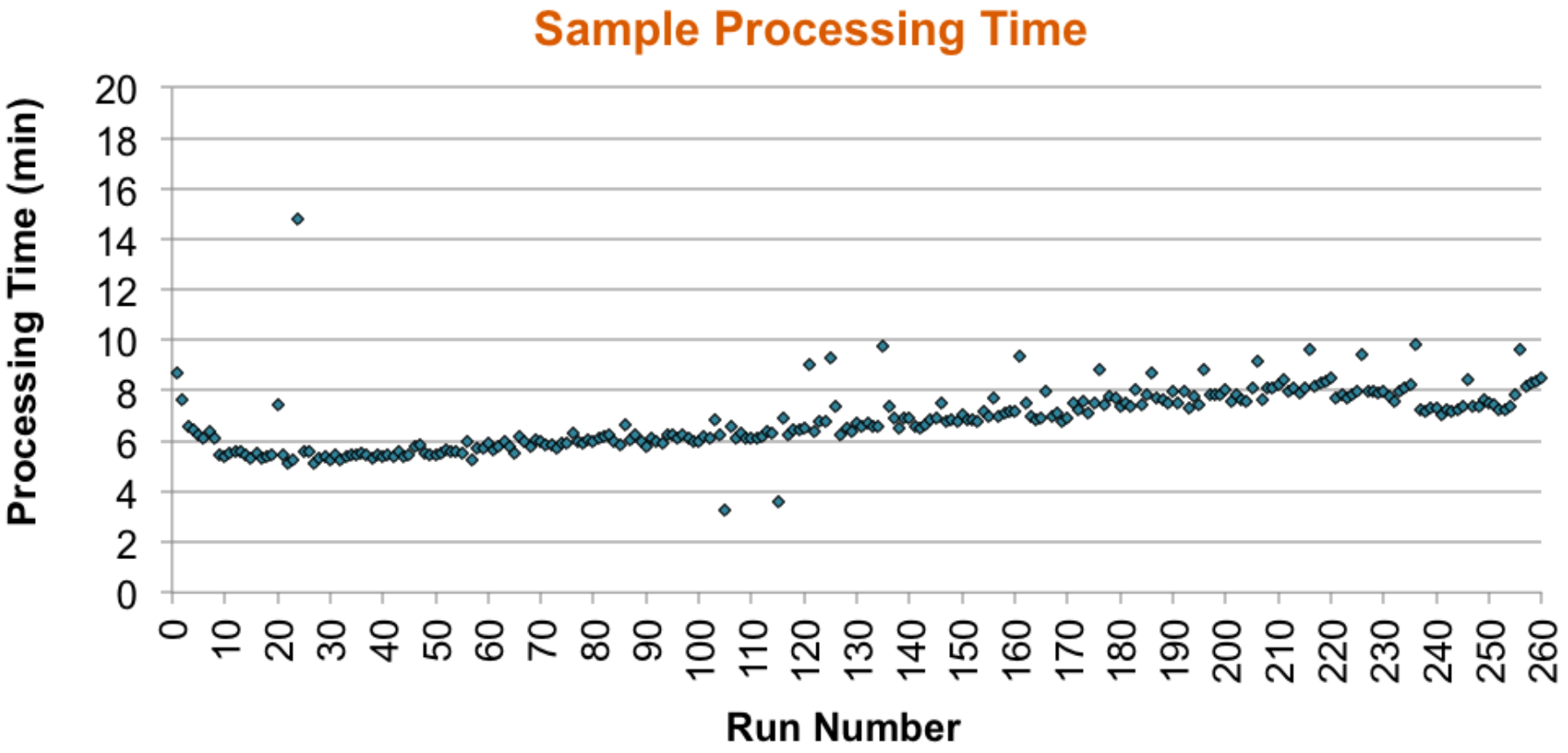
Integration with MFSI M-BAND

Microfluidic Systems Inc.'s (MFSI) ~~Microfluidic~~ Bioagent Networked Detector (M-BAND)

Interferent Testing



Interferent Testing



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