

A Novel Membrane-Based System for Fractionation and Concentration of Biological Particles from Complex Environmental Matrices

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October 19, 2012



DHS PHASE II SBIR

- Development of an automated, integratable sample fractionation/concentration system for use autonomous biodetection systems

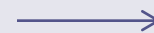


Autonomous Biodetection Systems

Aerosol
Collection



Sample
Preparation



Detection



- 5 to 20 mL
- Inhibitors
- Clutter



?



- 0.001 to 0.2 mL
- Bacteria
 - Culture
 - DNA
 - Proteins
 - Other
- Viruses
 - DNA/RNA
 - Proteins
- Free DNA
- Free Proteins

Autonomous Biodetection Systems

- Remove environmental debris
- Separate into 5 fractions
 - Whole bacteria
 - DNA from bacteria
 - Proteins from bacteria
 - Free DNA and Viruses
 - Free Proteins
- Concentrate from ~10 mL into 100 uL

Autonomous Biodetection Systems – System Goals

- Process 10 mL in 15 minutes or less
- Efficiently remove environmental debris (including humics) and clutter
- Concentrate 5 fractions into 100 uL volumes
- Desired Concentration Efficiency of 70% (50% acceptable)
- Operational for up to 30 Days (240 samples)

Technical Approach

Membrane Filter Fractionation & Concentration

6 membranes (minimum) + lysis

Cartridge 1

- 1 - Remove Environmental Debris
- 2 – Concentrate Whole Bacteria
- 3 – Concentrate Free DNA/Viruses
- 4 – Concentrate Free Proteins

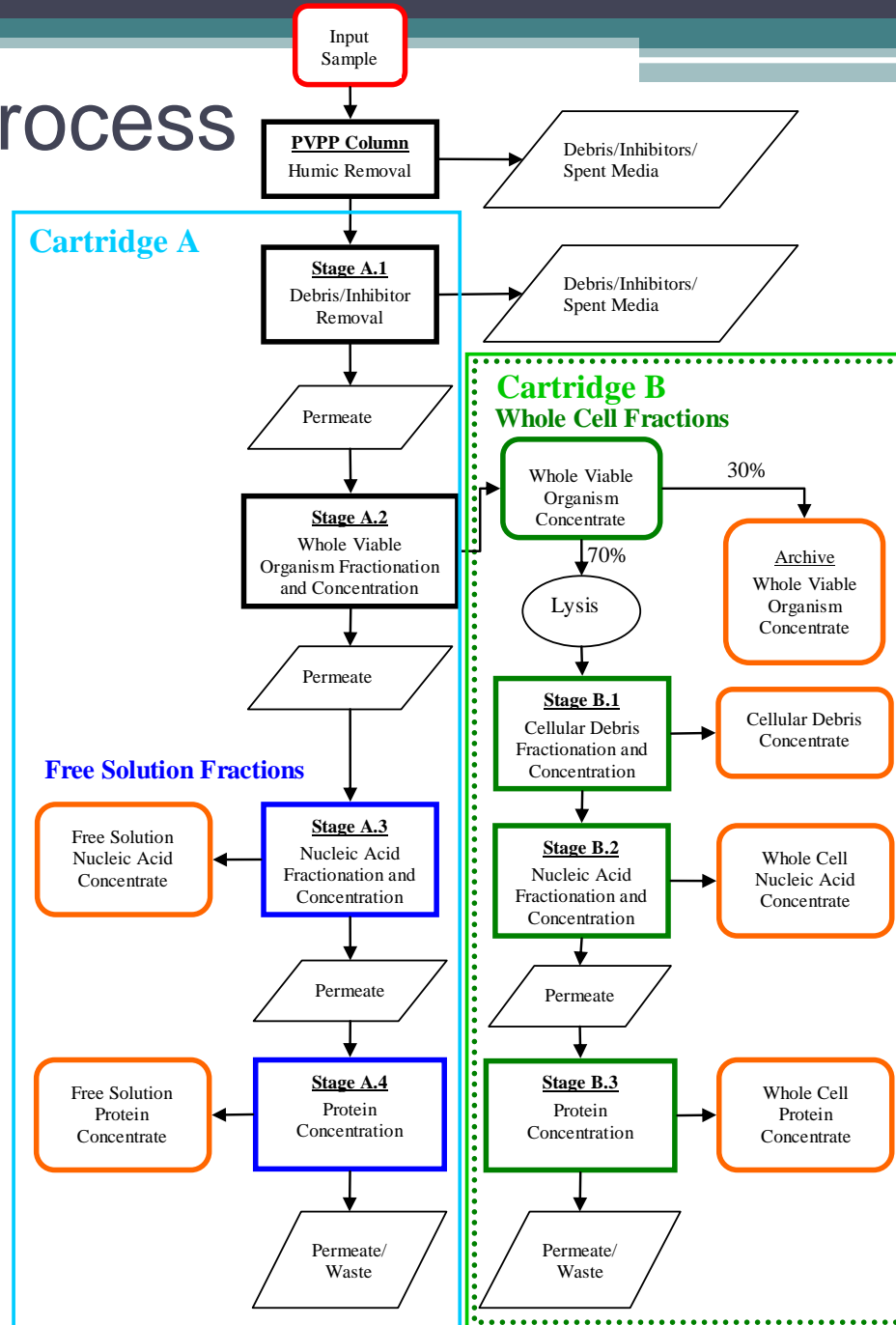
Lysis

- Lyse Whole Bacteria

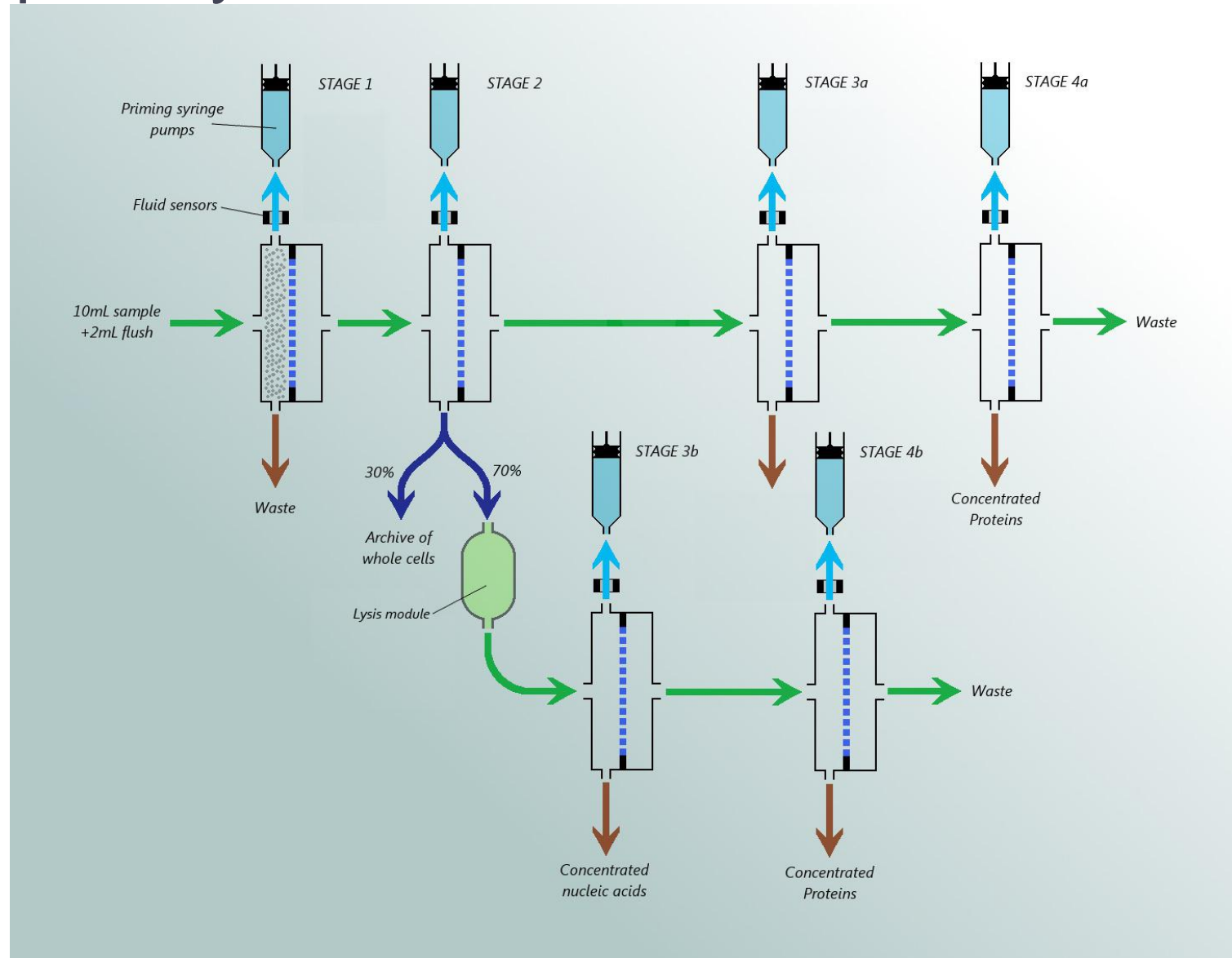
Cartridge 2

- 1 - Free DNA
- 2 - Free Proteins

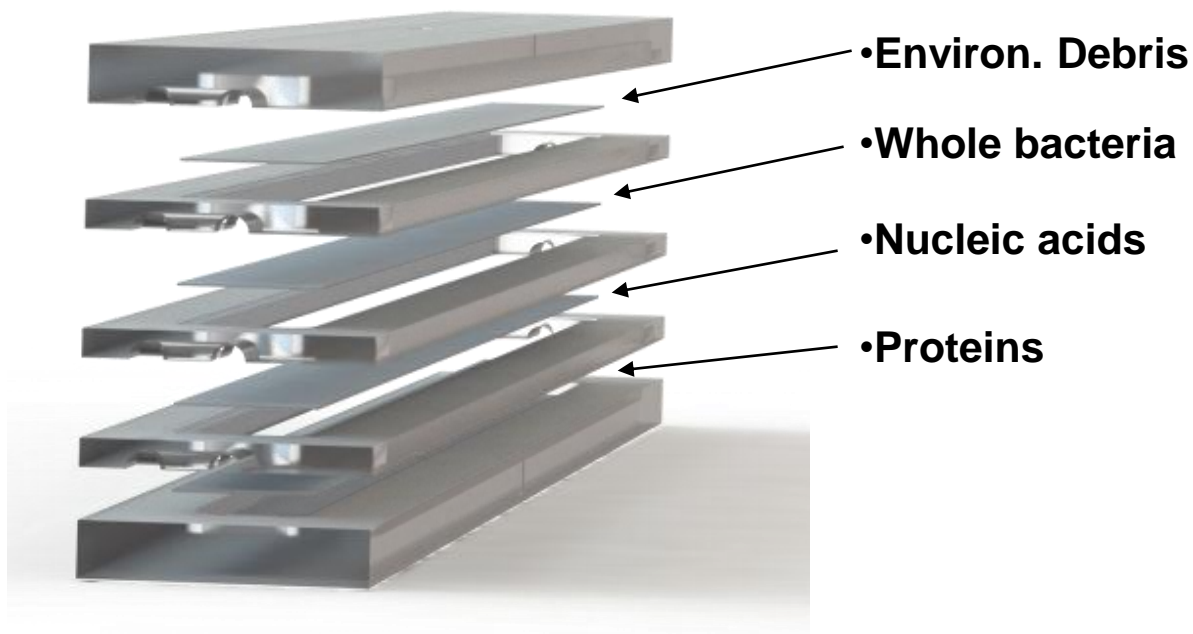
Complex Process



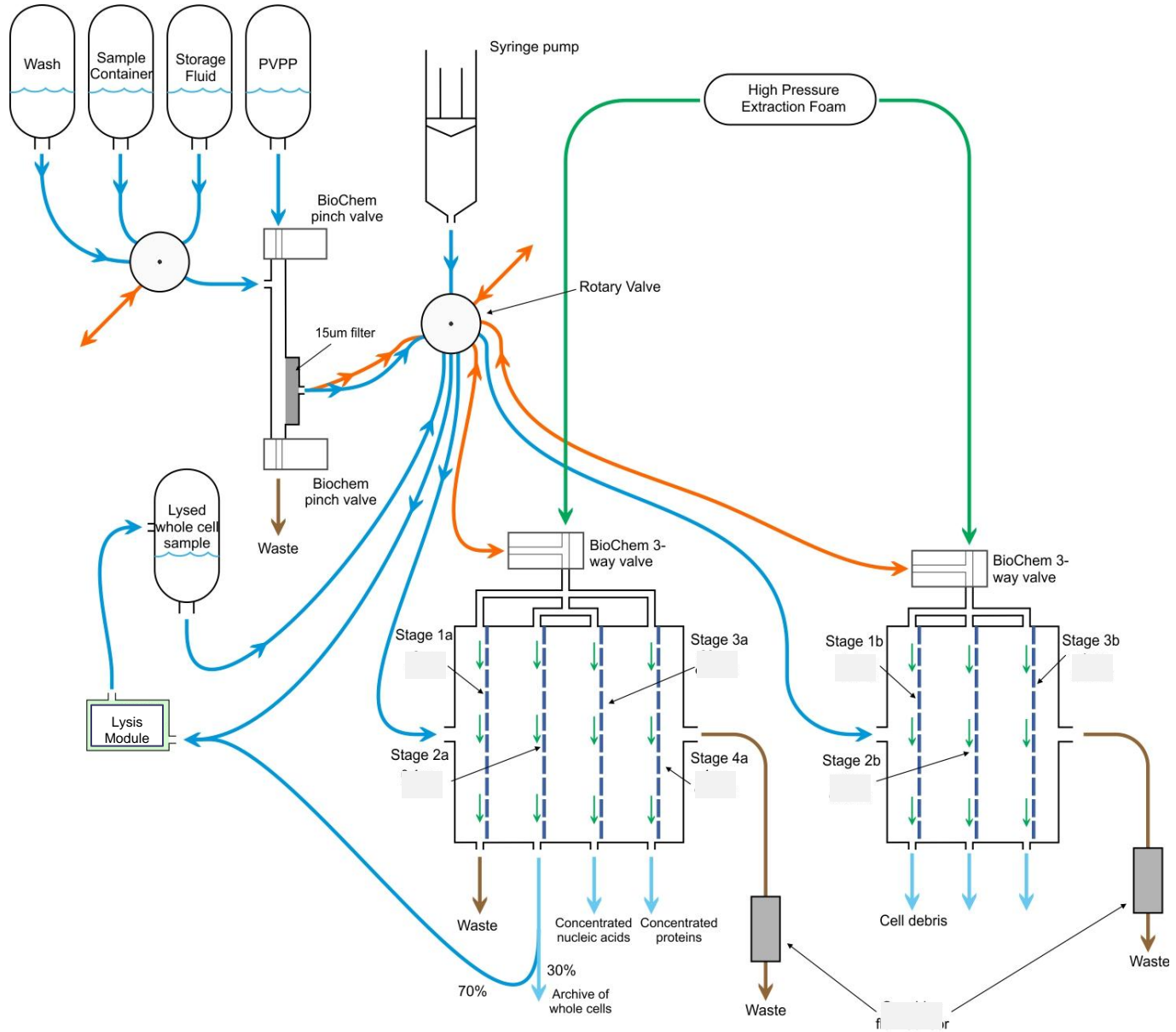
Complex System



Improved “Cartridge” Design



.....Still Complex



Concentration Process

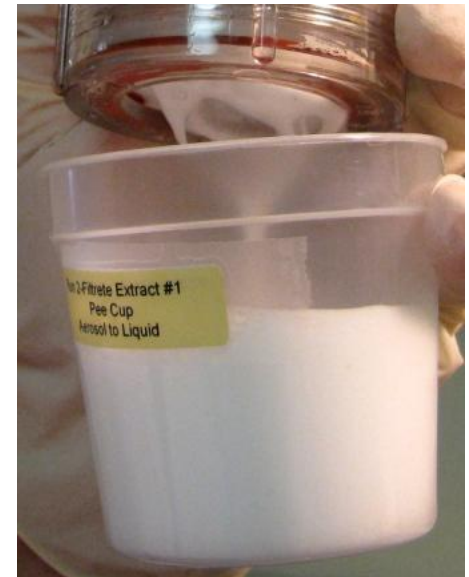


The InnovaPrep Concentration Process

- “Dead-end” filtration
- Particles larger than the filter pore size are retained
- A viscous, expanded wet foam is pushed tangential to the filter collection surface - capturing the particles

Wet Foam Elution

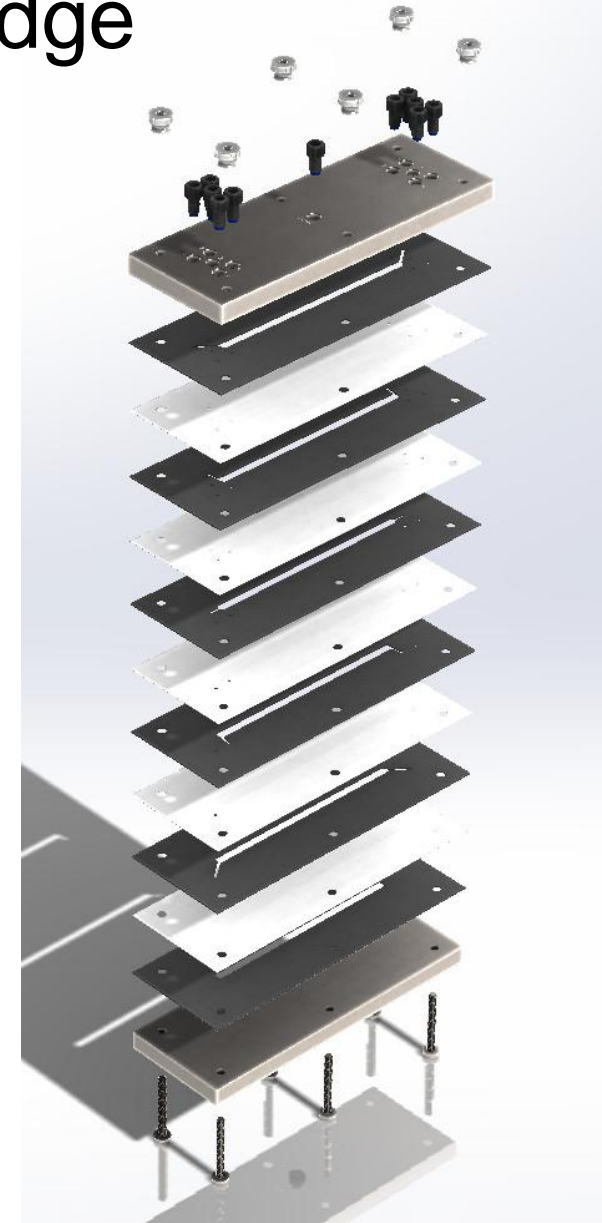
- Mode of Operation
 - “Expanded Liquid”
 - Increased Viscosity – reduced channeling (Yan 2006)
 - Moves as rigid body w/ narrow ($<10\ \mu\text{m}$) lubricating layer (Briceno 2003; Tisne 2004)
 - Bursting bubbles
- Improved Elution
 - Lower minimum elution volumes
 - Higher elution efficiencies
 - Largely unaffected by sample matrix
- Quickly Breaks Down into a Liquid



System Design

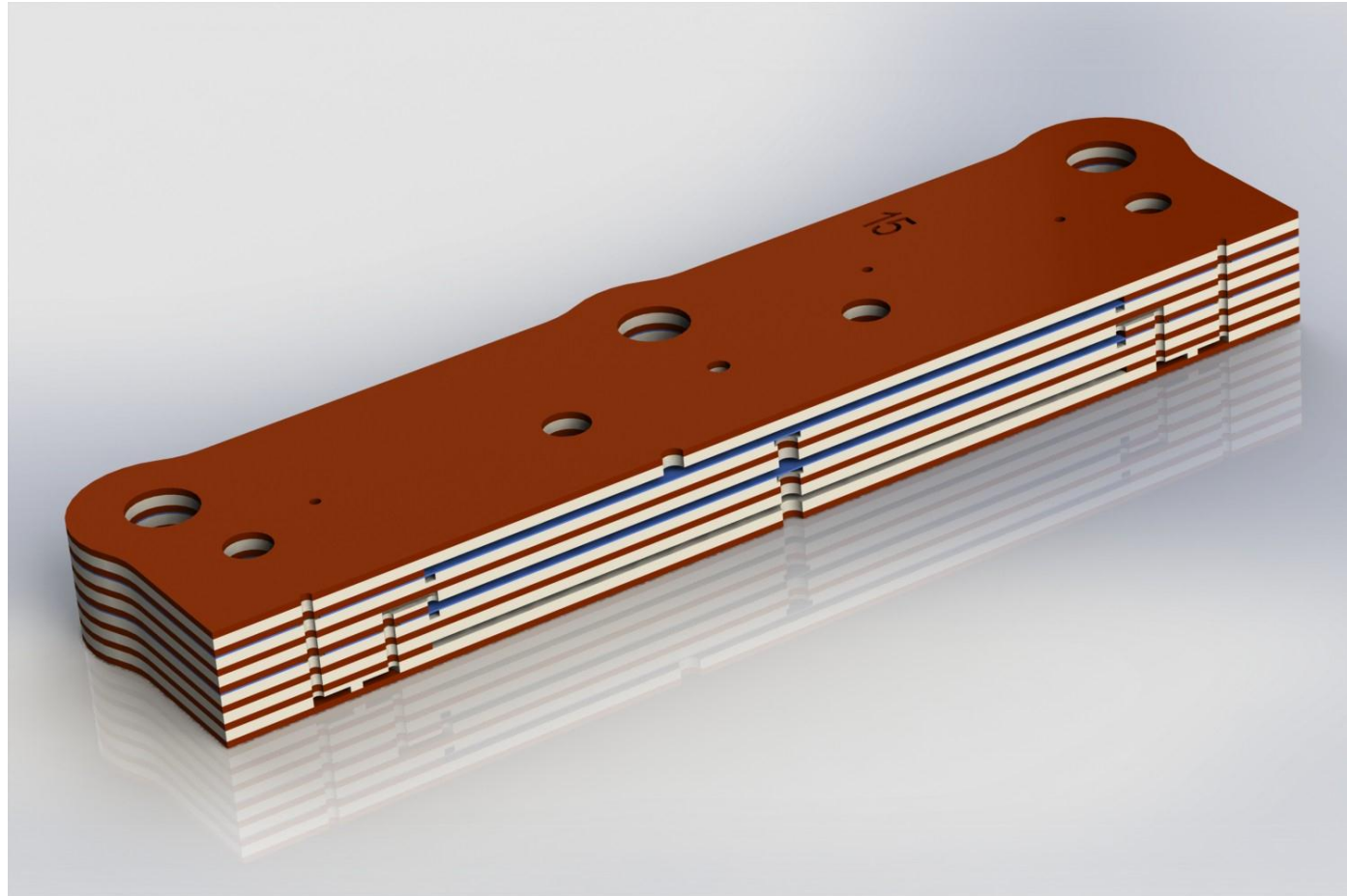
Membrane Filter Cascade Cartridge

- Stack of 5 membranes
 - Environmental Particle Debris
 - Humics
 - Whole Bacteria
 - DNA/Viruses
 - Proteins
- Flow Sequentially through Each Membrane
- Wet Foam Elution

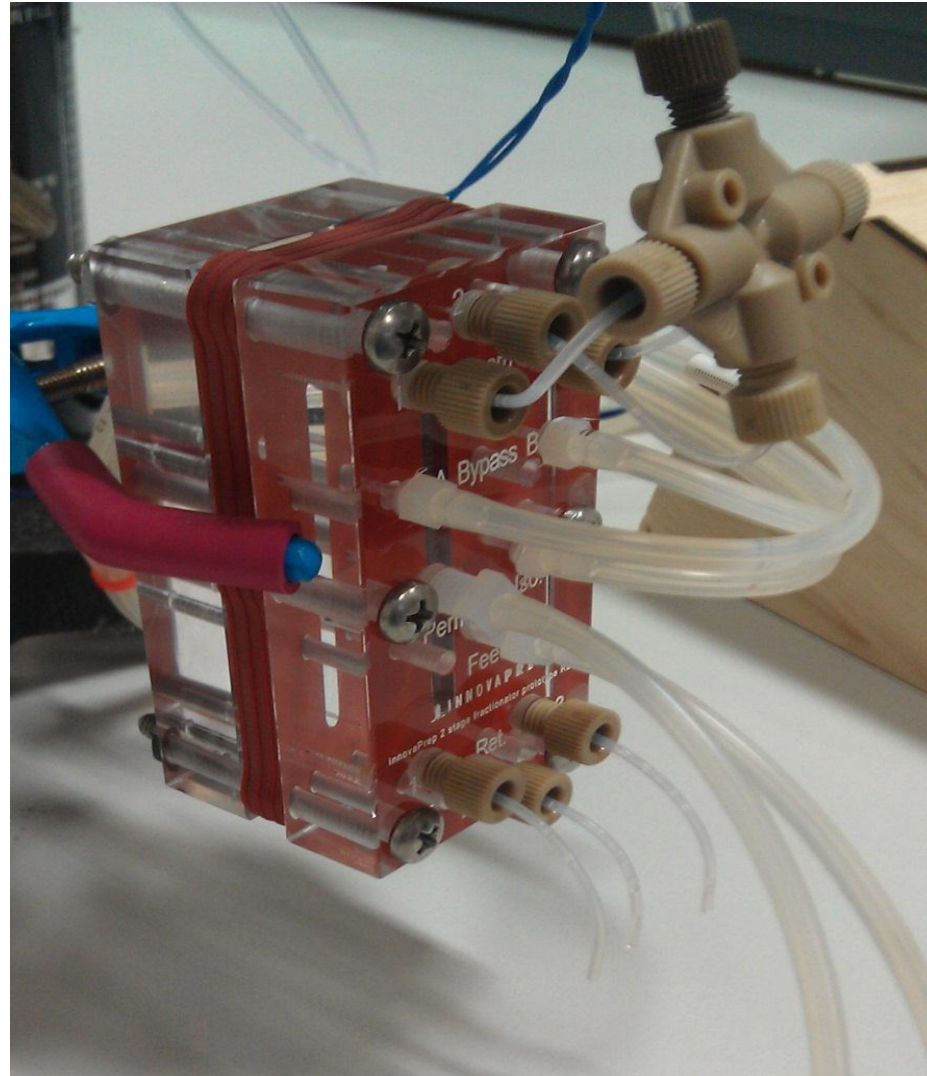


Membrane Filter Cascade Cartridge

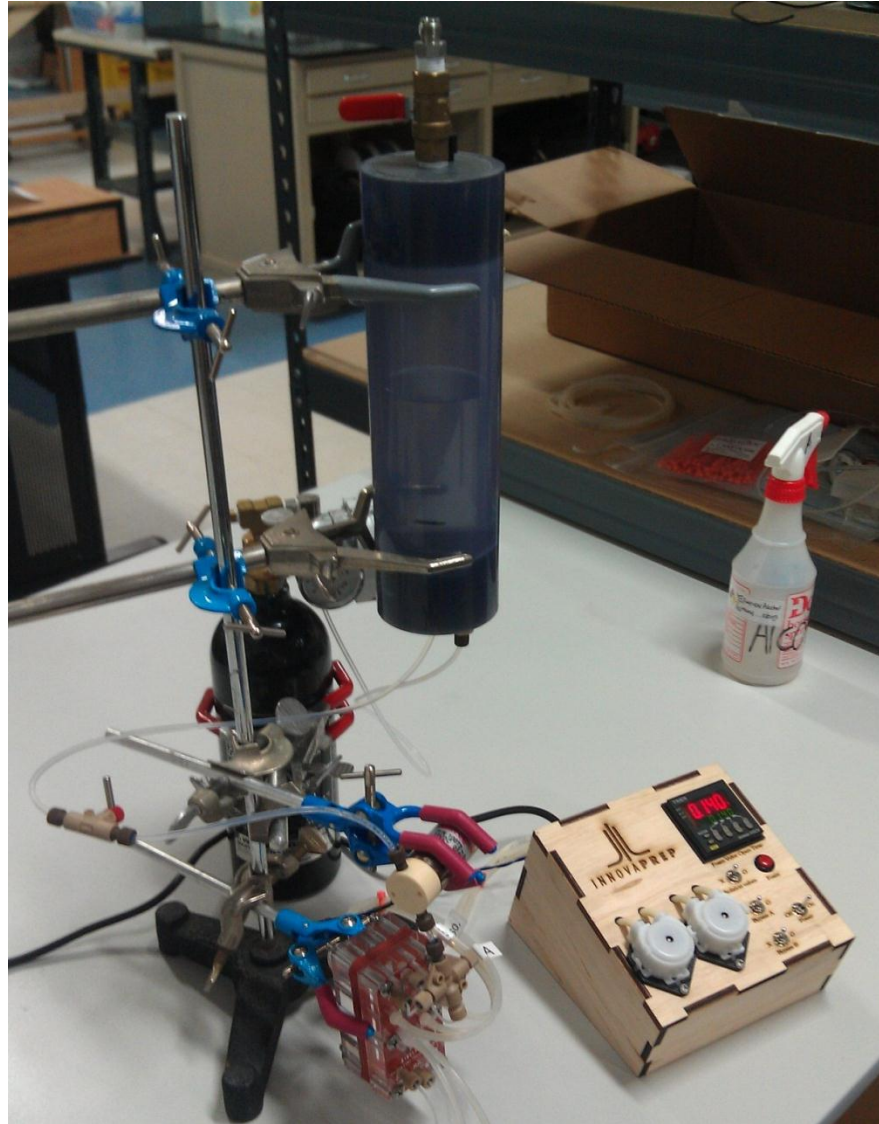
- Stack of 5 membranes
- Integrated Pneumatic Valves



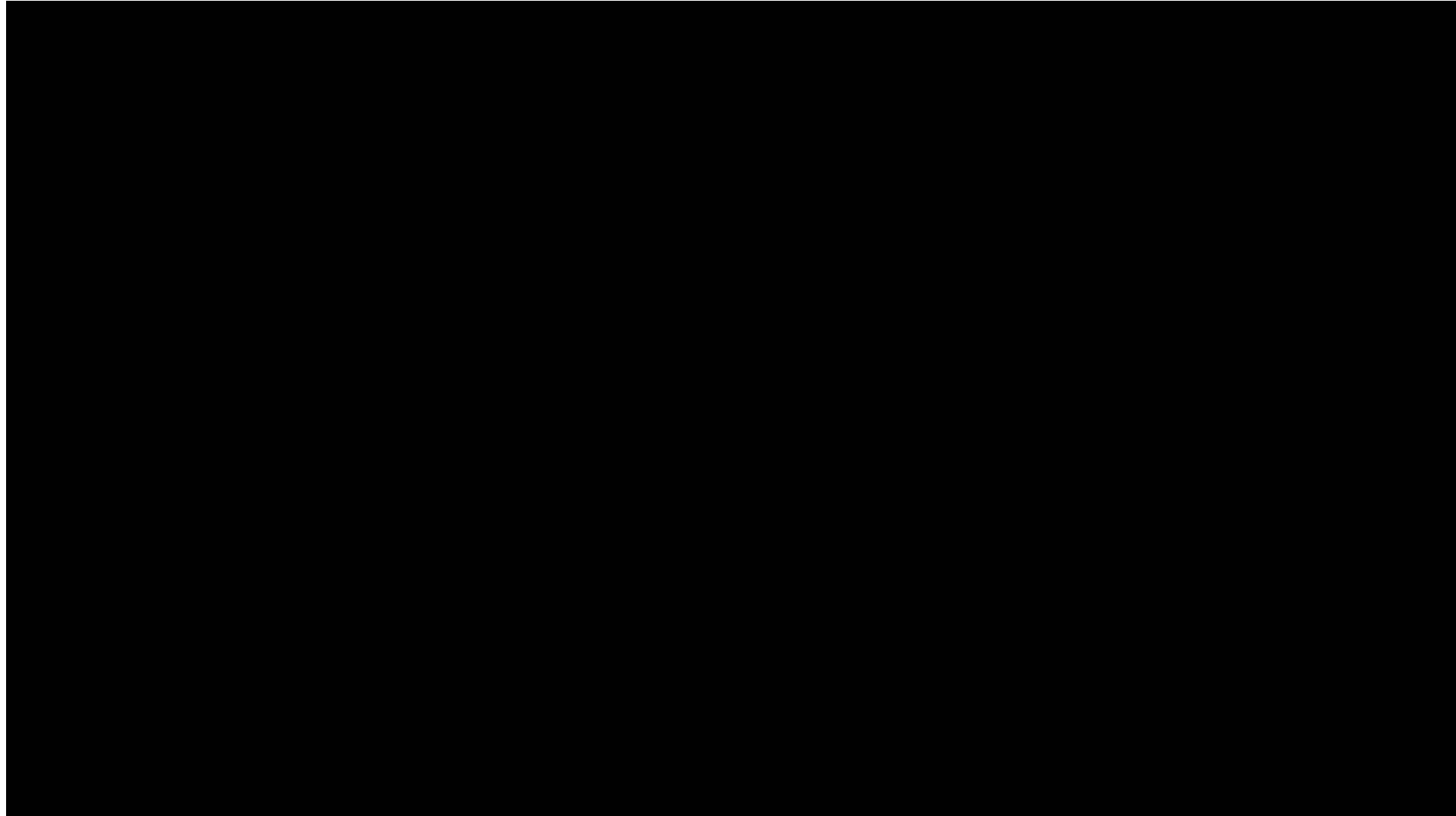
2-Stage Cartridge



Manual Test System w/ 2-Stage Cartridge



Video of 2-Stage Cartridge in Operation



2 Stage Results - 1 um Polystyrene Microspheres

- 10 mL sample volume, 5.0×10^7 beads
- Stage 1 – 10 um pore membrane
- Stage 2 – 0.4 um pore membrane

| Run Number | Run 10 | Run11 | Run 12 |
|---------------------------------|--------|-------|--------|
| Run Time, min | 1.55 | 1.60 | 1.38 |
| Stage 1 Elution 1 – 10 um | | | |
| • Elution Volume, μL | 94 | 71 | 81 |
| • Recovery Efficiency, % | -0.1% | 5.1% | 2.3% |
| Stage 2 Elution 1 – 0.4 um | | | |
| • Elution Volume, μL | 221 | 189 | 217 |
| • Recovery Efficiency, % | 87% | 83% | 85% |
| Permeate | | | |
| • Permeate Volume, mL | 9.3 | 9.7 | 9.6 |
| • Percentage to Permeate, % | 1.1% | 1.2% | 1.3% |
| Mass Balance, % | 90% | 90% | 90% |

Estimated System Efficiencies

| | Whole Bacteria | Free Solution Fractions | | | Whole Cell Fractions | |
|----------------------|----------------|-------------------------|-------|----------|----------------------|----------|
| | | Viruses | DNA | Proteins | DNA | Proteins |
| Humic/Debris Removal | 84.6% | 73.5% | 73.5% | 92.0% | 84.6% | 84.6% |
| Whole Cell A.2 | 83.9% | 84.6% | 84.6% | 93.1% | | |
| Lysis | | | | | 90% | 90% |
| Nucleic Acid A.3 | | 70.3% | 70.3% | 93.1% | | |
| Protein A.4 | | | | 72.2% | | |
| Nucleic Acid B.2 | | | | | 70.3% | 93.1% |
| Protein B.3 | | | | | | 72.2% |
| Full System | 71.0% | 43.7% | 43.7% | 57.6% | 53.5% | 51.2% |

Technical Hurdles

- Higher-Performance Membranes
 - Very High Flux
 - Highly Selective
- Membrane Fouling
 - Particles
 - Humics
- Flux/Transmembrane Pressure vs. Transmission
- System Complexity

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