

Novel Fluidics Microbead Trap/Flow Cell Enhances Speed/Sensitivity of Bead-Based Bioassays 5-Fold

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Abstract

Automated purification and concentration of cells, nucleic acids, and proteins is critical to enable trace detection in environmental, clinical and food samples. Automation improves reproducibility, allows for unattended operation/monitoring and can actually improve analytical performance relative to assays conducted in a batch mode.

Pacific Northwest National Laboratory (PNNL) has developed a variety of fluidics devices and methods for automating sample preparation and detection. At the heart of these devices is a unique micro/nano particle trap (Enhanced Bead Assay Device) that allows surface-functionalized magnetic or non-magnetic particles to be trapped with subsequent perfusion of sample, reagents and wash solutions. This approach can yield significant (>5-fold) improvements in assay speed and sensitivity, while significantly reducing sample matrix effects.

Results are presented that highlight the analytical performance improvements obtained for automated microbead assays utilizing PNNL's microbead trap/flow-cells relative to assays conducted in a more traditional "batch mode" (i.e., in tubes or microwell plates).

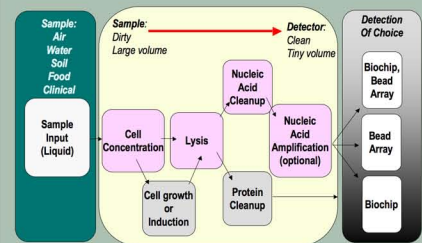
Introduction

Challenges for Detection in Complex Samples

- Variable background
- Background interferes with detection
- Problems with accuracy
- Traditional sensor approaches have limited system lifetimes
- Small amounts of pathogens can cause illness:
Example: 1 pathogen/mL
 - 1 analyte per pathogen: 1.7 zM (1.7×10^{-21} M)
 - 1000 analytes per pathogen: 1.7 aM (1.7×10^{-18} M)

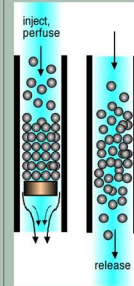
➡ **Need Purification AND Concentration**

Automated Purification and Concentration of Cells, Biomolecules, and Chemicals



Functionalized Microbeads Enable Purification and Concentration

Beads with interactive surfaces for selective capture of cells, biomolecules, chemicals, radionuclides

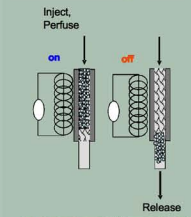


- Beads automatically delivered and released
 - 5-150 μ m nonmagnetic particles (polymer, hydrogel, glass, etc., spherical or non-spherical)
 - 50 nm-10 μ m magnetic particles
- Can reuse beads, then automatically replace, as needed
- Compatible with sample processing 'unit operations'
- Operates within a scaleable fluidics architecture:
Nanoliter to Liter Volumes

Microbead Trap/Flow Cell Advantages

- Enhanced Mass Transport
 - Lower Limits of Detection for Assays
 - Faster reaction kinetics/higher throughput
- Efficient/Complete Washing
 - Rapidly Remove Interfering Matrix/Inhibitors
 - Minimize False Positives/Negatives
- Microbeads Automatically Delivered/Released
- Microbead Columns can be Pre-Made as Disposable Cartridges
- Up to 12 Sample Parallel Processing
Design Adaptable to 96-Well Microplates

Electromagnetic Microbead Trap

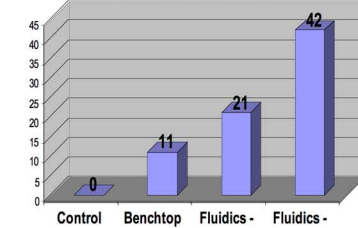


Nickel Foam

- Highly Porous
- Captures Microparticles Uniformly Across Flow Channel

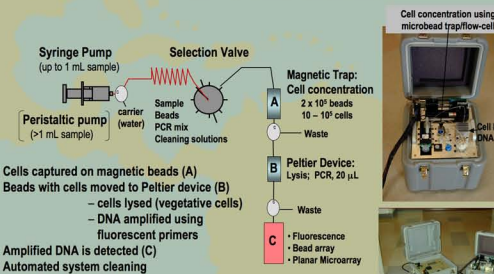
E. coli 0157:H7 Capture Using Antibody-Coupled Magnetic Beads

% Capture of 2000 CFU/mL E. coli Using Magnetic Particle Trap/Flow Cell vs. Standard Approaches in 50 mL Sample Volumes, 1 hr capture time



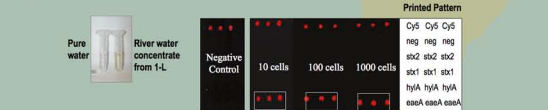
Magnetic Microbead Trap Gives 4X Higher Cell Capture Efficiency

E. coli Capture, Purification, Lysis and Flow-Through PCR with Microarray Detection



- Cells captured on magnetic beads (A)
- Beads with cells moved to Peltier device (B)
 - cells lysed (vegetative cells)
 - DNA amplified using fluorescent primers
- Amplified DNA is detected (C)
- Automated system cleaning

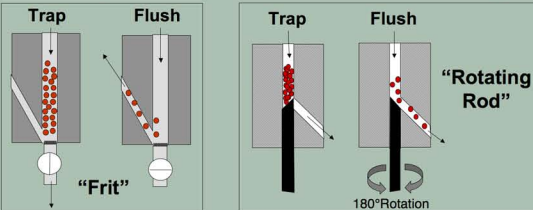
Reproducible detection of 10 cells/mL in river water concentrate



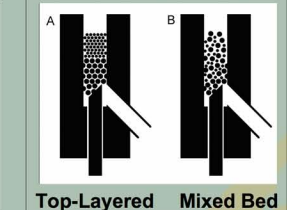
Without sample prep: PCR was inhibited, resulting in false negatives

Magnetic Microbead Trap Allows Complete and Rapid Removal of PCR Inhibitors

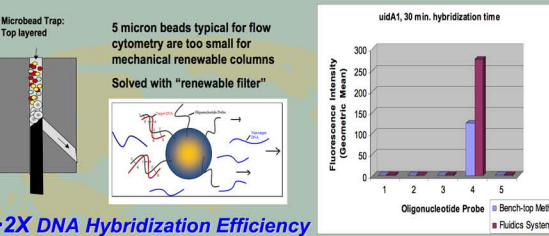
Non-Magnetic Microbead Trap/Flow Cell Designs



Bead Packing Strategies

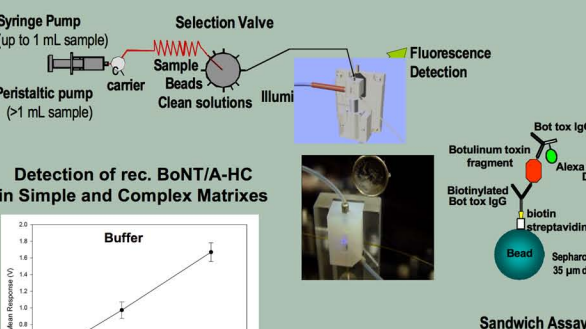


DNA Hybridization on Heated Microbead Column

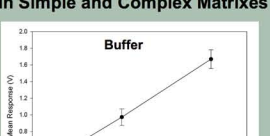


>2X DNA Hybridization Efficiency

Direct Fluorescence Detection of Sandwich Assay on Microbead Column

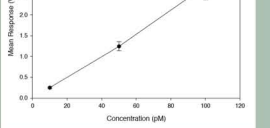


Detection of rec. BoNT/A-HC in Simple and Complex Matrixes



Lowest detected: 10 pM
Lowest tested: 1 pM in bkgrnd
Goal \rightarrow 0.04 ng/mL or 1 pM

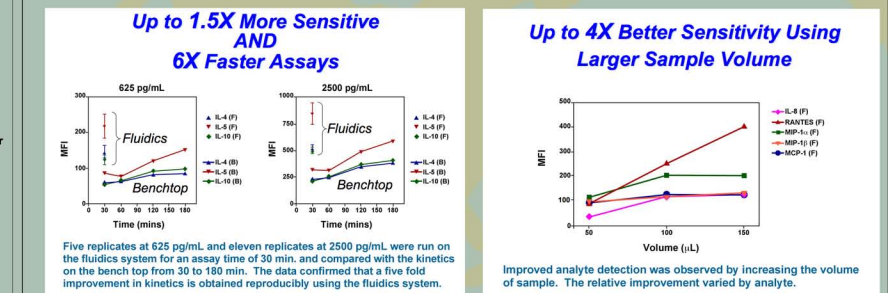
FBS-Fetal Bovine Serum



FBS provides a complex protein rich model system in which to detect our target

10 Picomolar Protein Detection in 15 minutes

5-Plex On-Column Cytokine Sandwich Immunoassay w/ Flow Cytometry Detection



Contributors: S Iyer, Ember, J. Bowman B., BD Biosciences

Summary

- Microbead trap handles magnetic and non-magnetic microparticles of various shapes, sizes, material types
- Flow through design well-suited to automated platforms
- Renewable or disposable design
- Single channel, multi-channel (up to 12), or 96-well plate compatible
- Scalable to process nanoliter to liter sample volumes
- Provides sample concentration AND purification
- Flexible/modular platform
 - Change bead types/surface chemistry for analysis of different organisms, biomolecules, chemicals (including radionuclides)
 - Include only modules needed for particular application (e.g., cell capture)
- Can link to multiple detector types (integrate or downstream measurement)

Microbead trap/flow cell improves sensitivity, speed, throughput

Acknowledgments

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