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Microfluidic Integration for Detection of Biomarkers of Infectious Diseases

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Team

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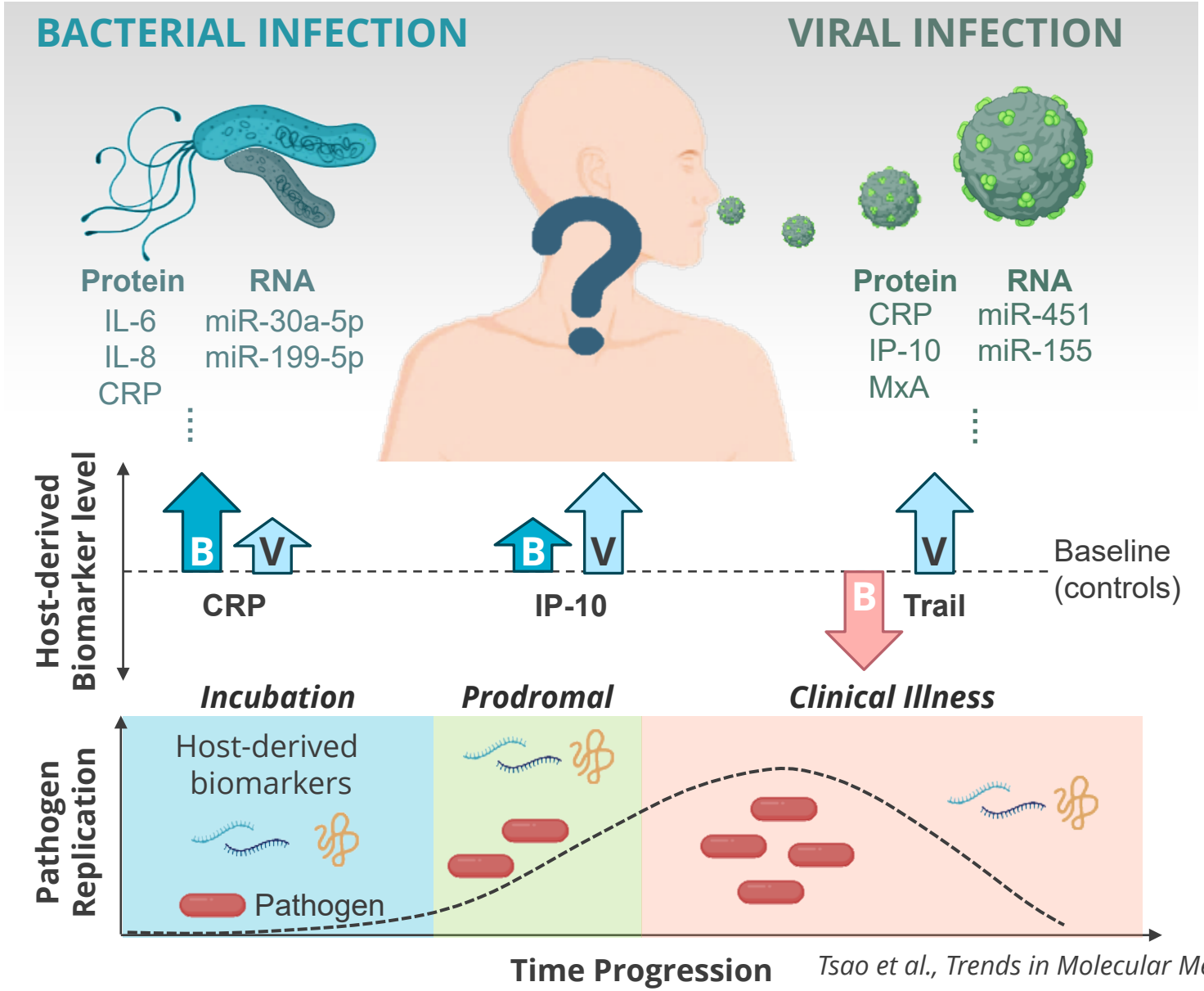
Outline

- In honor of Victor Ugaz
- Motivation: Role of Protein & RNA Biomarkers in Diagnostics
- Fieldable Biomarker Testing: Needs and Challenges
- Overview of microfluidic immunoassay device
- Microfluidic integration strategies for multimode analysis
- Amplification-free molecular assay for RNA biomarkers detection
- Conclusion

Protein & RNA Biomarkers as Early Indicators of Infection



Tsao et al., Trends in Molecular Medicine, 2020

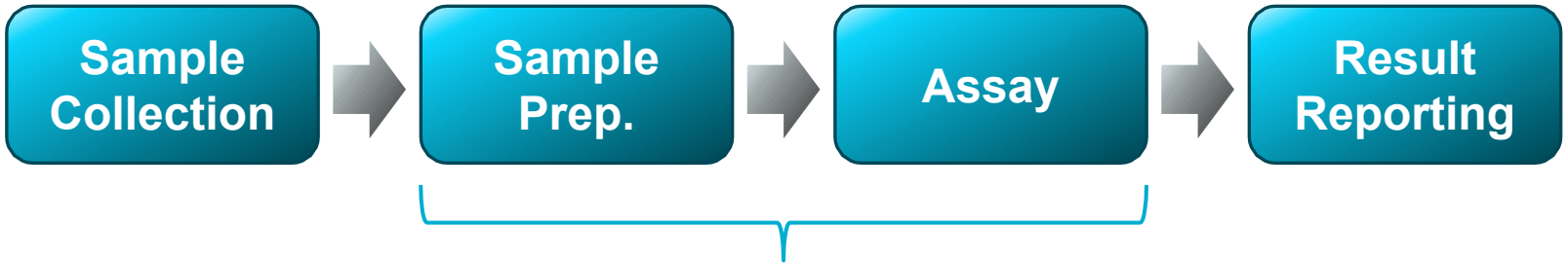
Tribolet el al., Frontier in Microbiology, 2020

Fieldable Biomarker Testing: Needs and Challenges

❑ Needs

- Rapid & cost-effective
- Sample stability
- Frequent tests
- Timely diagnosis

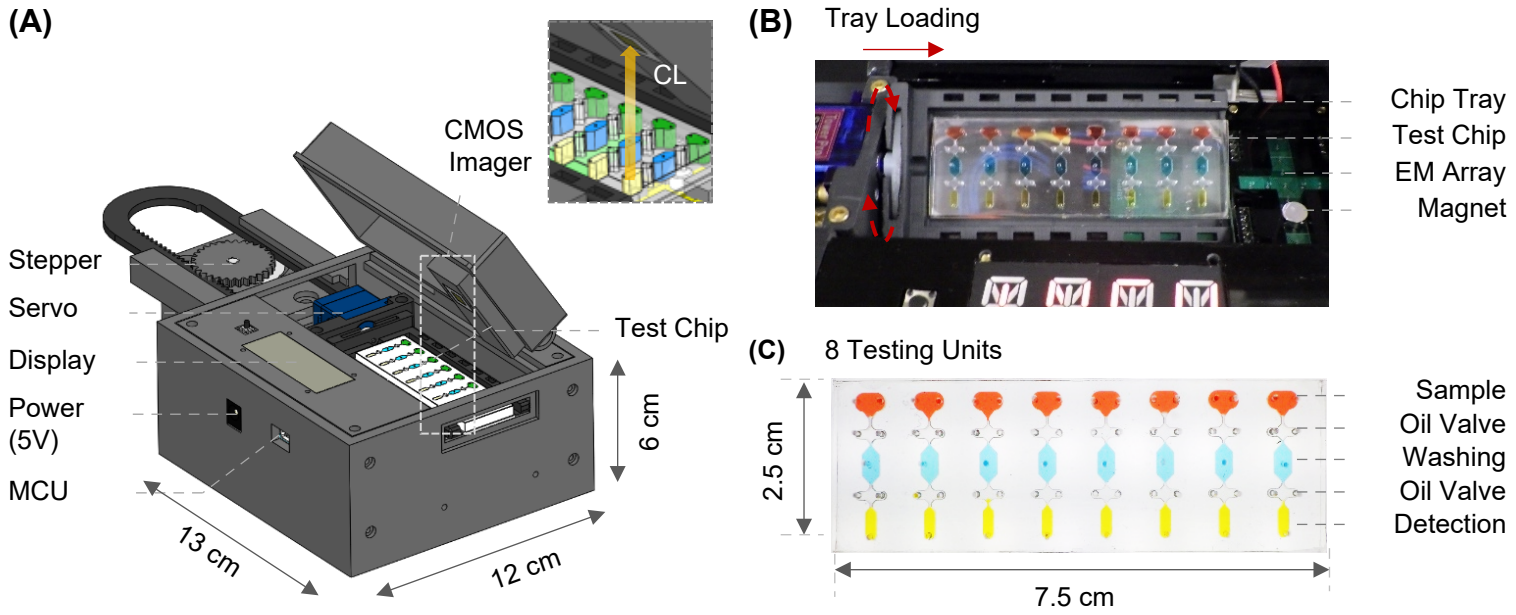
❑ Challenges



- Requirement for bulky instrumentation and/or multiple manual steps are hurdles to perform point-of-care biomarker analysis.
- Proteins and RNA require different assay workflow / instruments
- Each biomarker has different dynamic range & LOD requirements

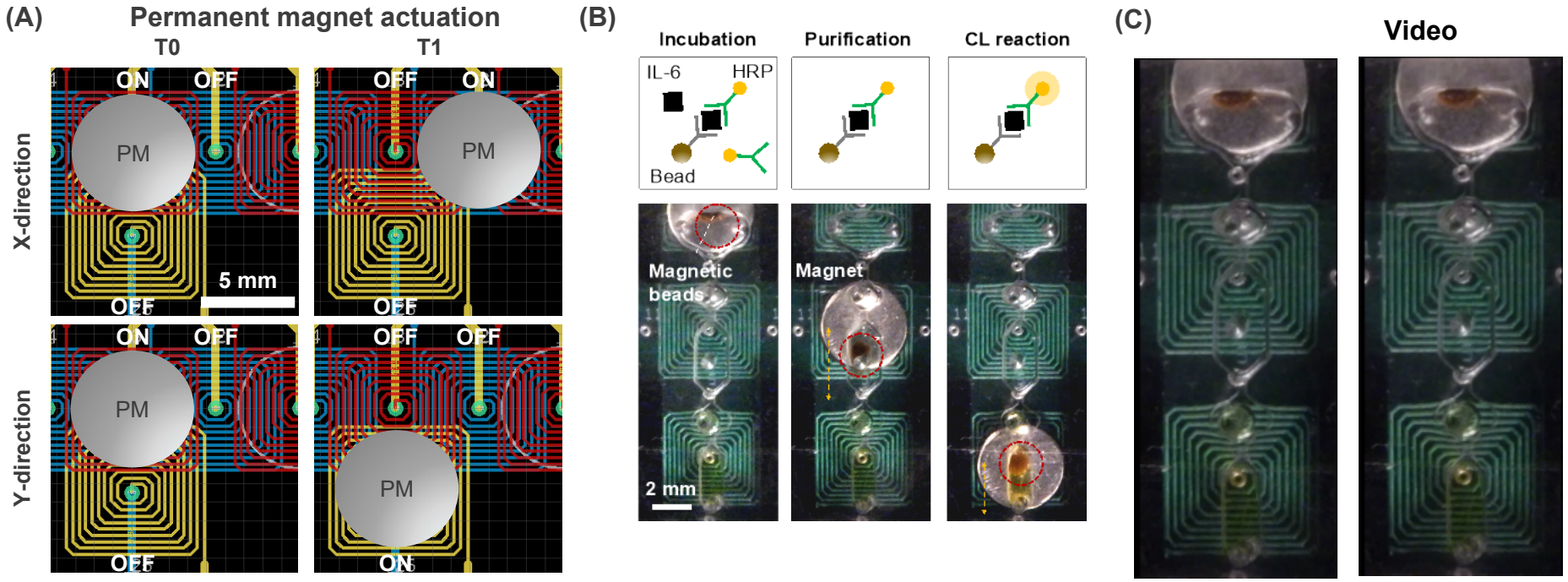
Microfluidic Immunoassay Device For Protein Biomarkers

First generation prototype (Choi *et al*, *Sensors & Diagnostics* 2024)



- Platform size: 13(l) x 12(w) x 6(h) cm
- Subsystem: CMOS image sensor, EM array, microcontroller, and display
- Attributes: Portable, automated, and tests 8 samples in 40 minutes

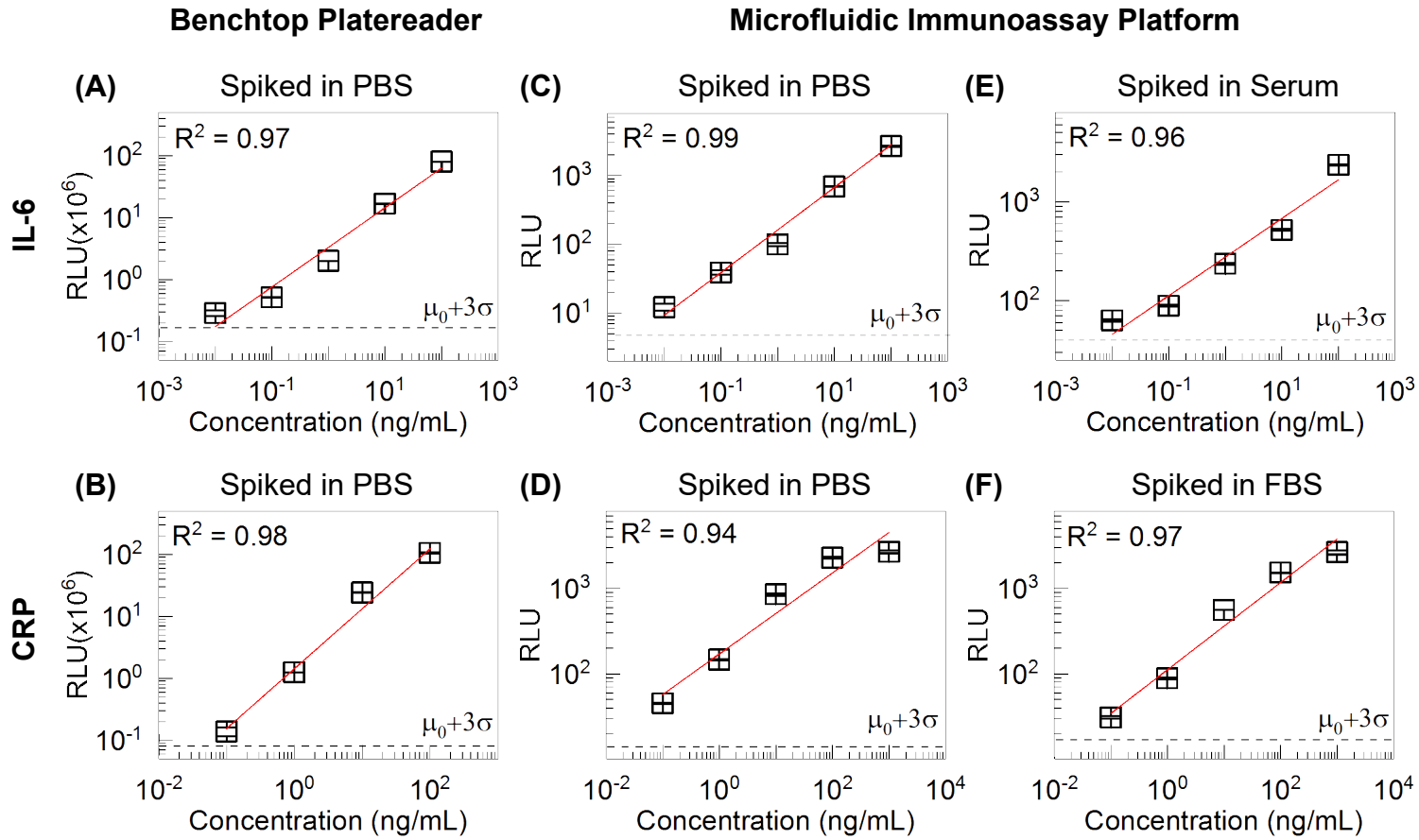
Integration Strategy 1: EM Array For Bead Actuation



Choi et al., Sensors & Diagnostics , 2024

- **Electromagnetic array**: overlapping planar coils on a PCB allows us to “amplify” a weak magnetic field from the coils by attracting a strong permanent magnet.
- Programmable actuation allows us to perform a multi-step assay procedure (e.g., binding, washing, detection)
- Implemented for bead-based sandwich immunoassays for protein biomarkers, with chemiluminescent readout

Sensitivity Validation on Cytokine Biomarkers

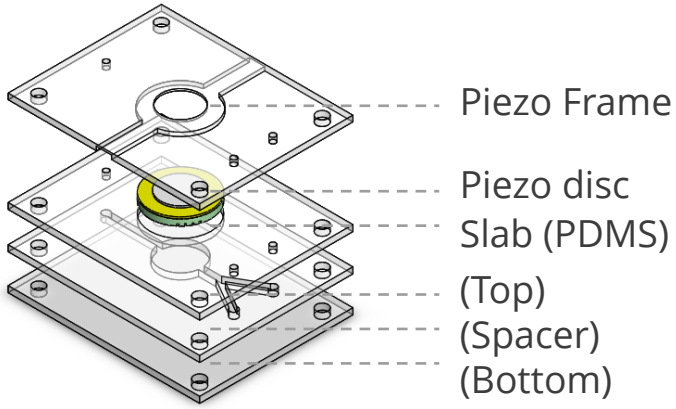


Choi et al., Sensors & Diagnostics , 2024

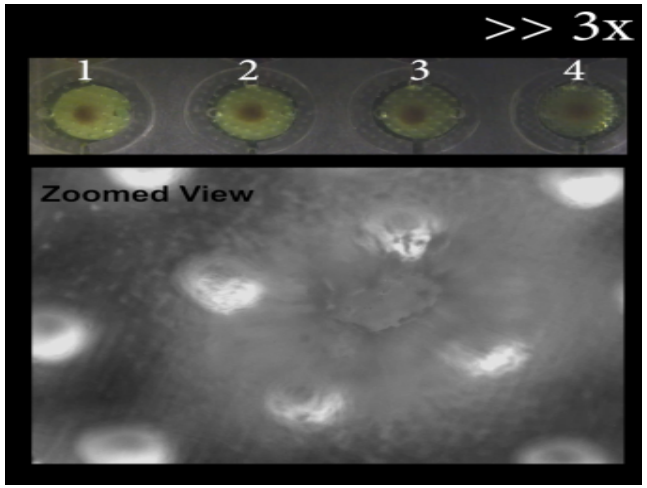
- Achieved detection sensitivity at physiologically relevant levels, with chip-based results in same range as a plate reader (10 - 100 pg/mL)
- No interference from sample matrix effect (human serum and fatal bovine serum)
- Device has ability to perform a qualitative as well as quantitative analysis

Integration Strategy 2: Acoustic Mixer For Reagent Mixing

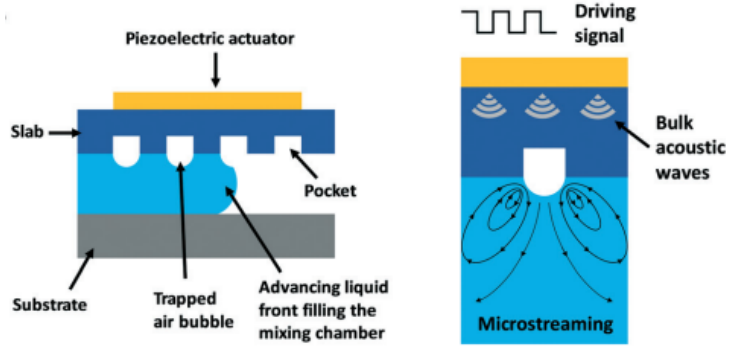
(A) Exploded view



(B) Bead suspension

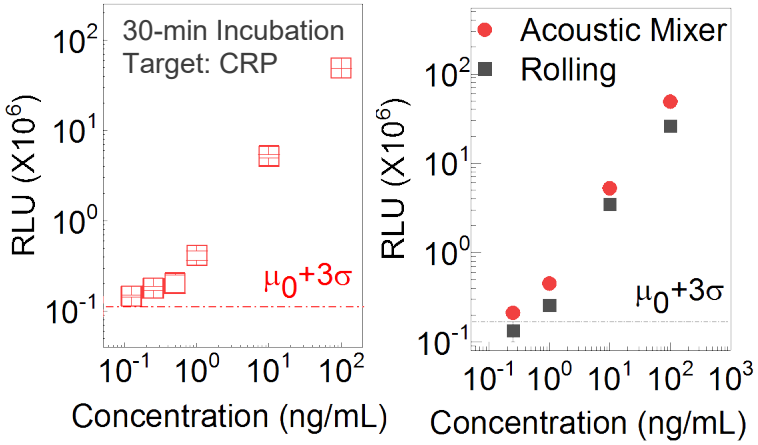


(C) Mixing mechanism

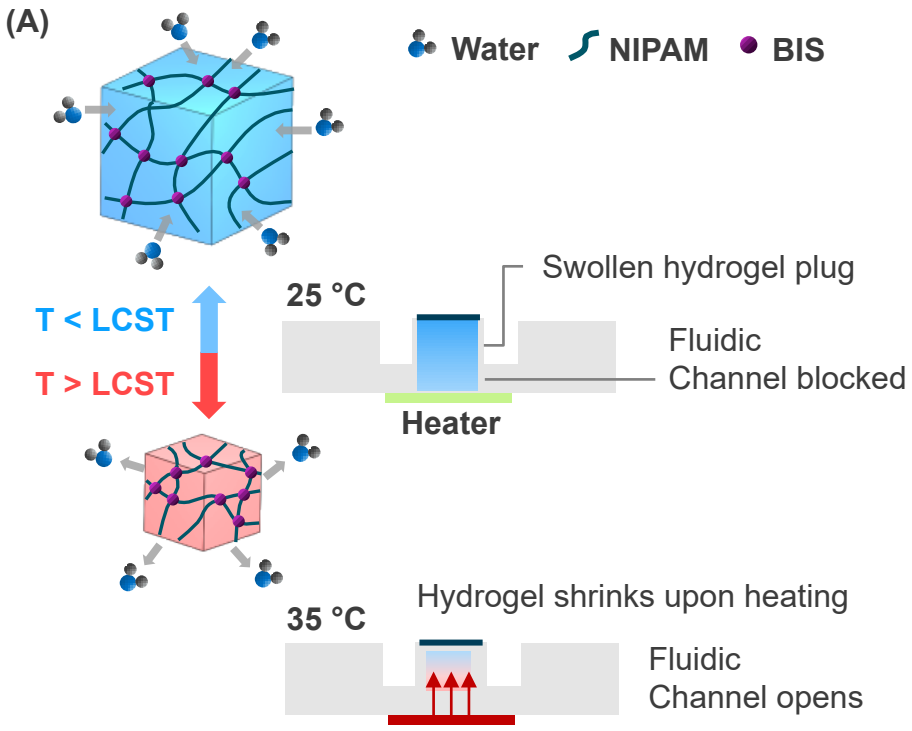


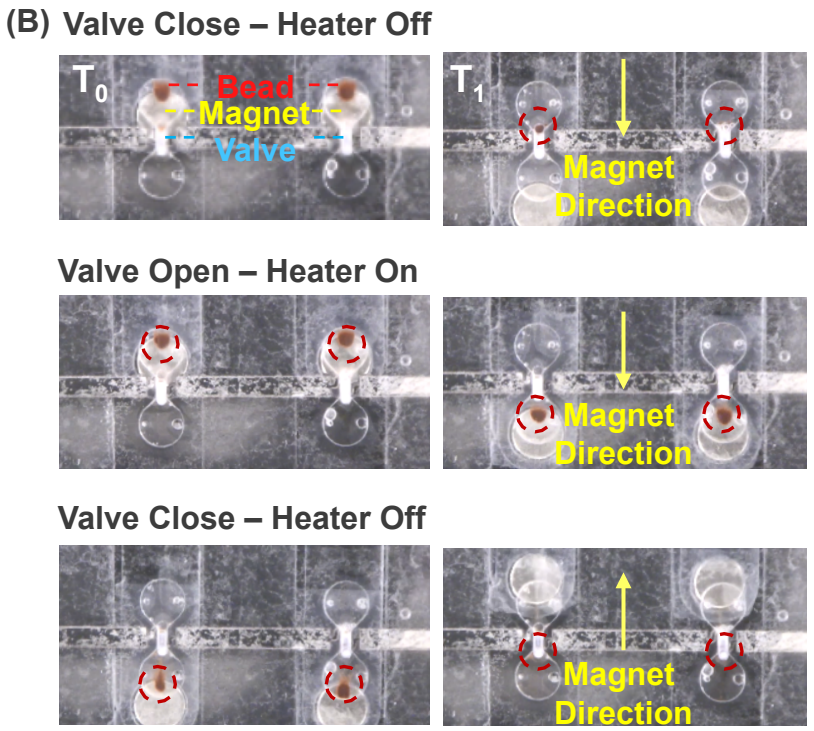
Conde et al., Lab Chip, 2020

(D) Target binding



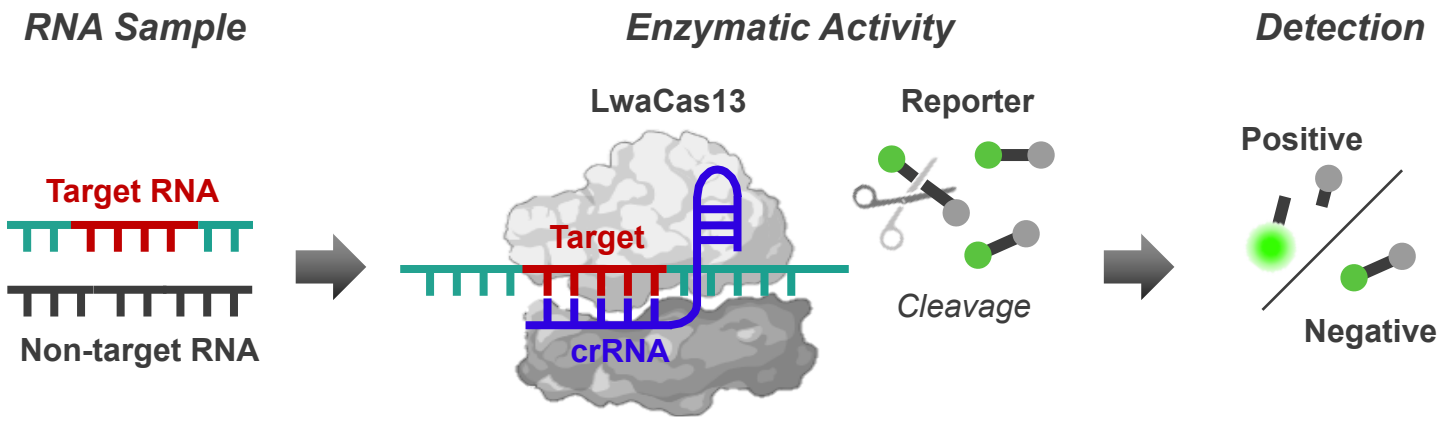
Microfluidic Integration 3: Thermally Responsive Hydrogel Valve to segregate reagents





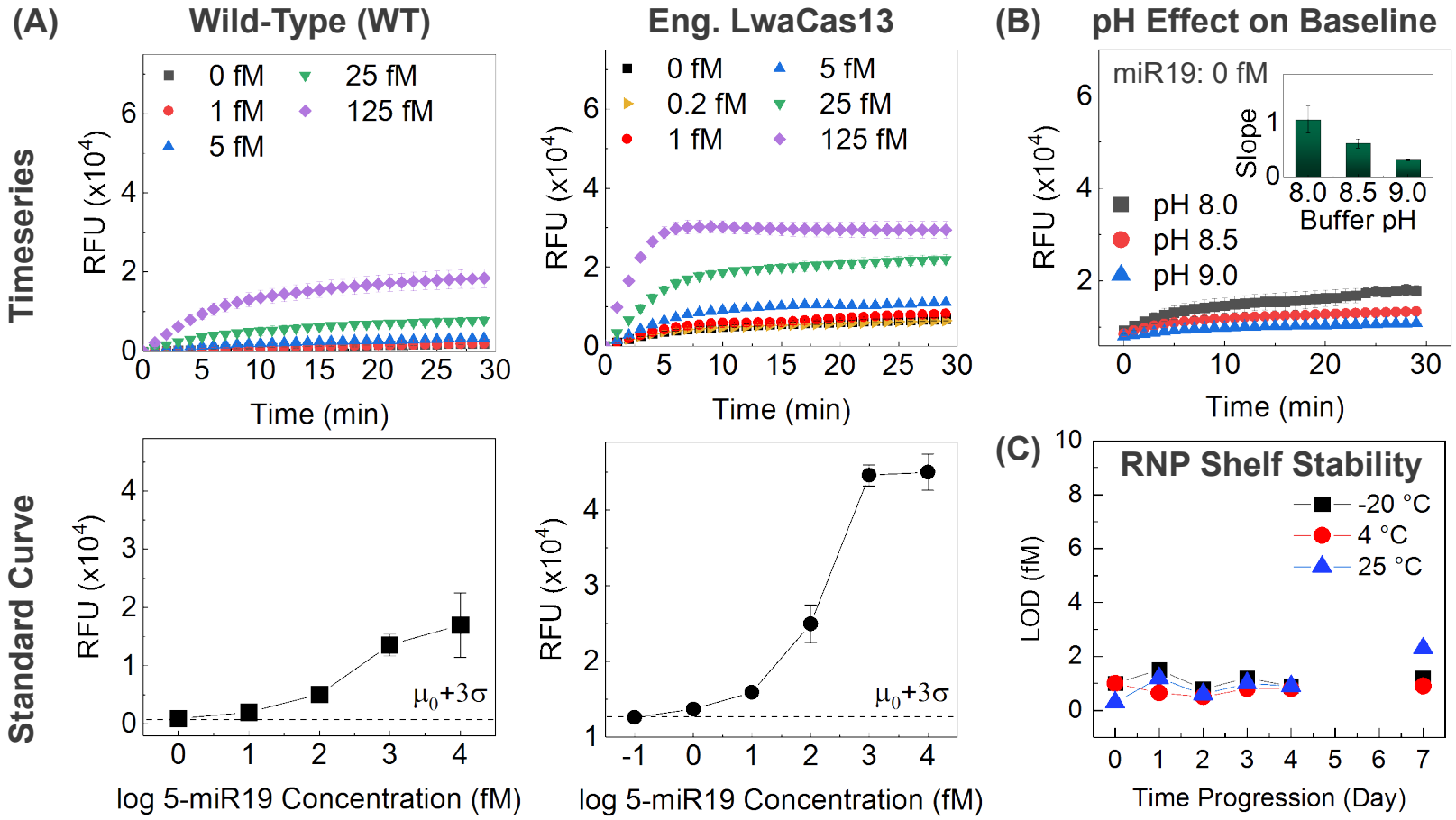
- Microfluidic valve can be electronically controlled using a resistive-heating element.
- Beads pass through the channel only when it's "open", at biocompatible temperature.
- Easier setup, less messy, and no bead loss at interface, vs oil "valve" used previously

Cas13-based Amplification-free RNA Detection Assay



- LwaCas13-crRNA RNP complex binding target RNA, resulting in activation of the HEPN nuclease domain (molecular scissors) , cutting the oligo reporters.
  - Cas13 detection is typically combined with target preamplification to achieve high sensitivity.
  - But target pre-amplification makes quantitation of the target difficult.
- Recent publication (*Yang et al., Nature Chemical Biology 2023*) reported that LwaCas13 with engineered RNA binding domain could detect RNA targets with attomolar sensitivity without amplification
- Enables quantitative analysis

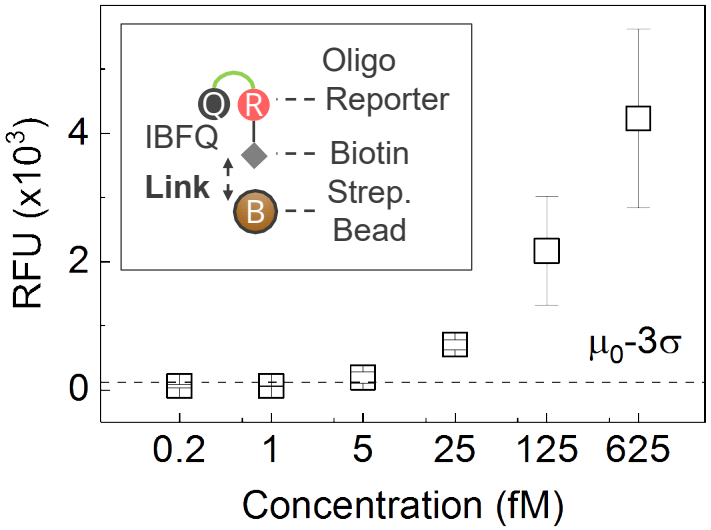
Optimization of Amplification-free Cas13-based RNA Detection



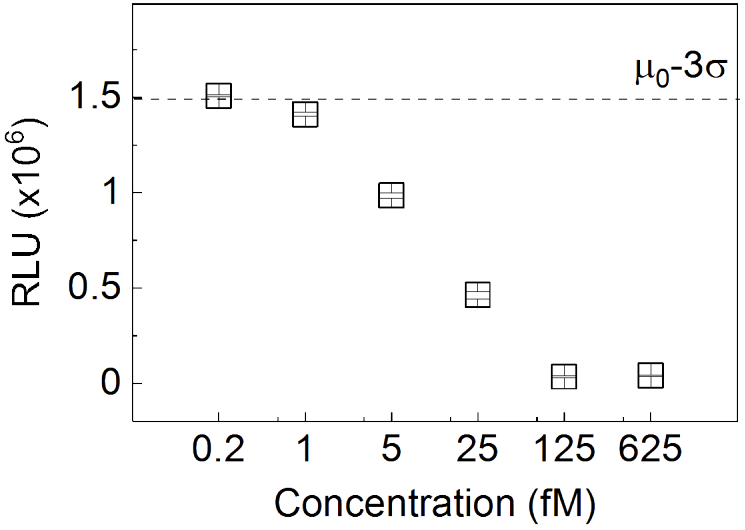
- Engineered LwaCas13 showed enhanced assay sensitivity (<1 fM) compared to WT in plate-based assay format.
- Reaction buffer pH contributes to suppress the background enzymatic activity.
- Shelf-stability of the Ribonuclear protein (RNP) is excellent (Ideal for fieldable application).

Translation of Cas13-based Detection into Bead-Based Assay

Magnetic Bead FL Reporter



Magnetic Bead CL Reporter



- Bead-based fluorescence (FL) and chemiluminescence (CL) reporters are compatible for Cas13 enzymatic activity.
- Sensitivity results with CL reporter outperforms FL reporter, but in a “signal-off” format
- The results with bead-based CL reporters showed comparable sensitivity to solution-phase RNA detection assay > suitable for integration on our platform.

Conclusion

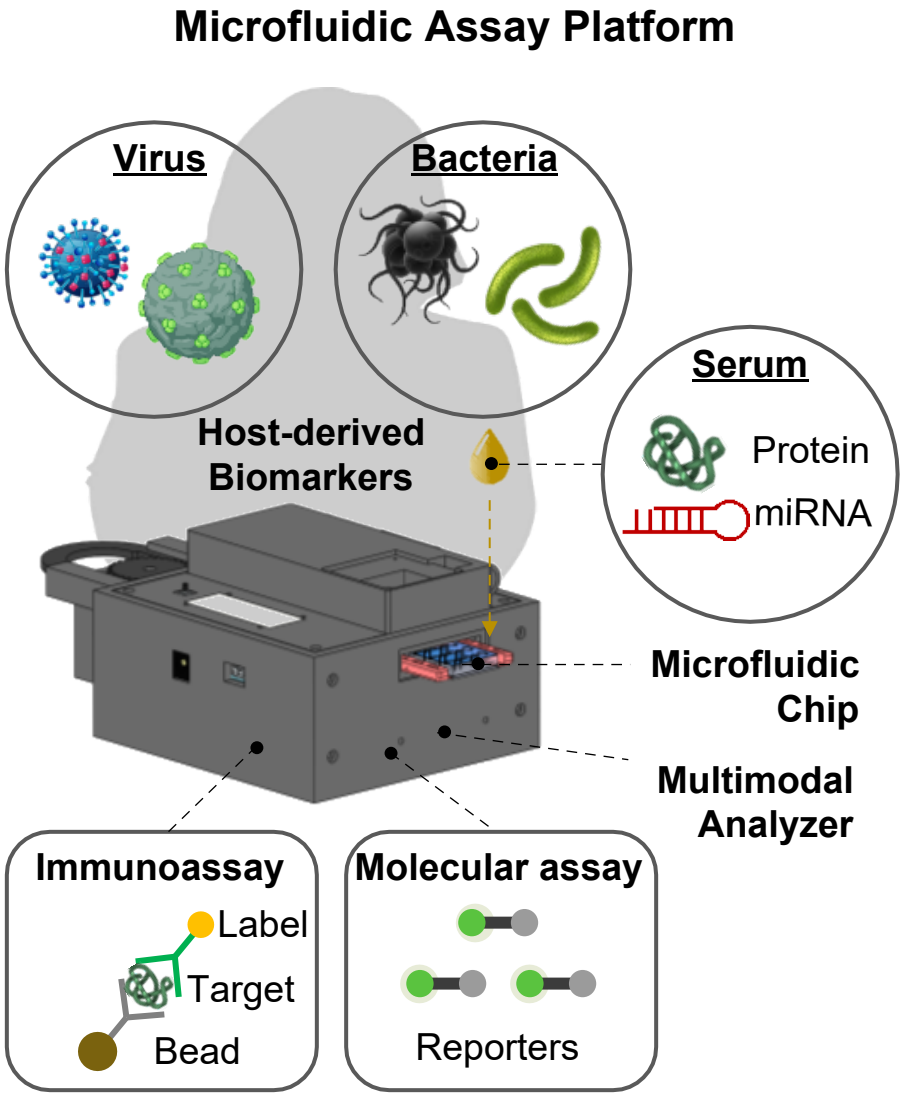
- A novel approach to microfluidic integration of assays for protein/RNA biomarker analysis
- A portable and easy-to-use instrument suitable for on-site detection of biomarker panels
- Microfluidic integration approaches allow automation of fundamental bead processing including binding, washing, detection, mixing, and valving
- Achieved 10-1000 pg/mL sensitivity with pro-inflammatory cytokine biomarkers at physiologically relevant levels
- Investigated bead-based approach to amplification-free detection of miRNA biomarkers with preliminary results in the low femtomolar range
- Currently integrating into a second-generation portable prototype adaptable for both cytokine and miRNA biomarker assays

THANK YOU

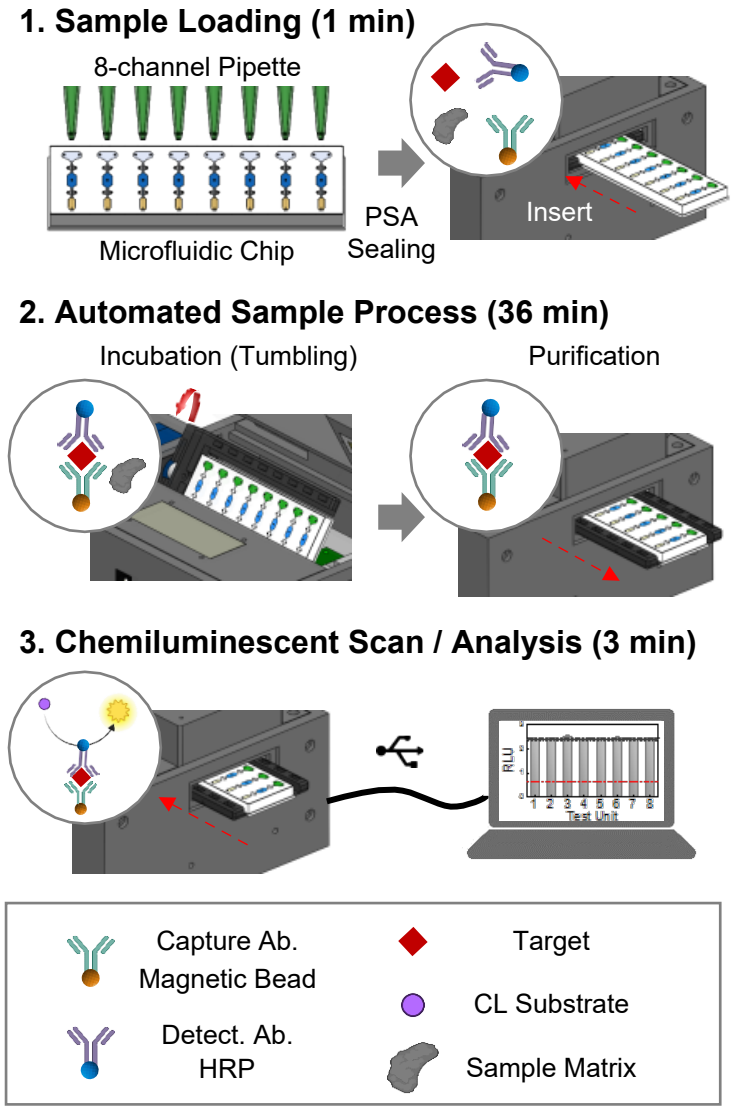
Contact: Robert Meagher, rmeaghe@sandia.gov

Additional material

Microfluidic Integration for Detection of Biomarkers

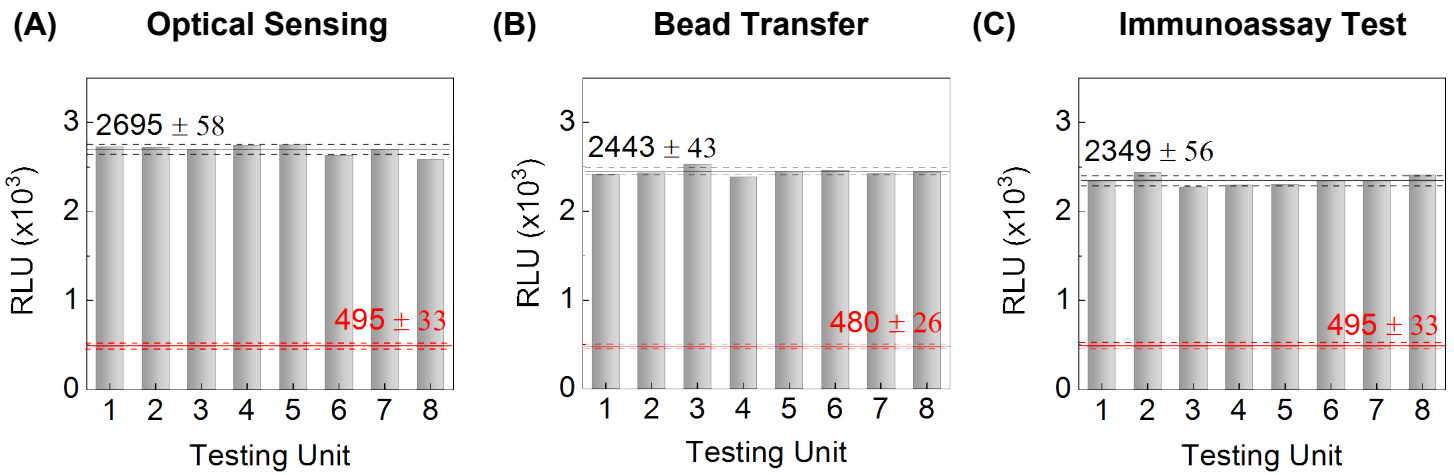


Sample Workflow



Choi et al., Sensors & Diagnostics , 2024

Unit-to-unit Consistency



Choi et al., Sensors & Diagnostics , 2024

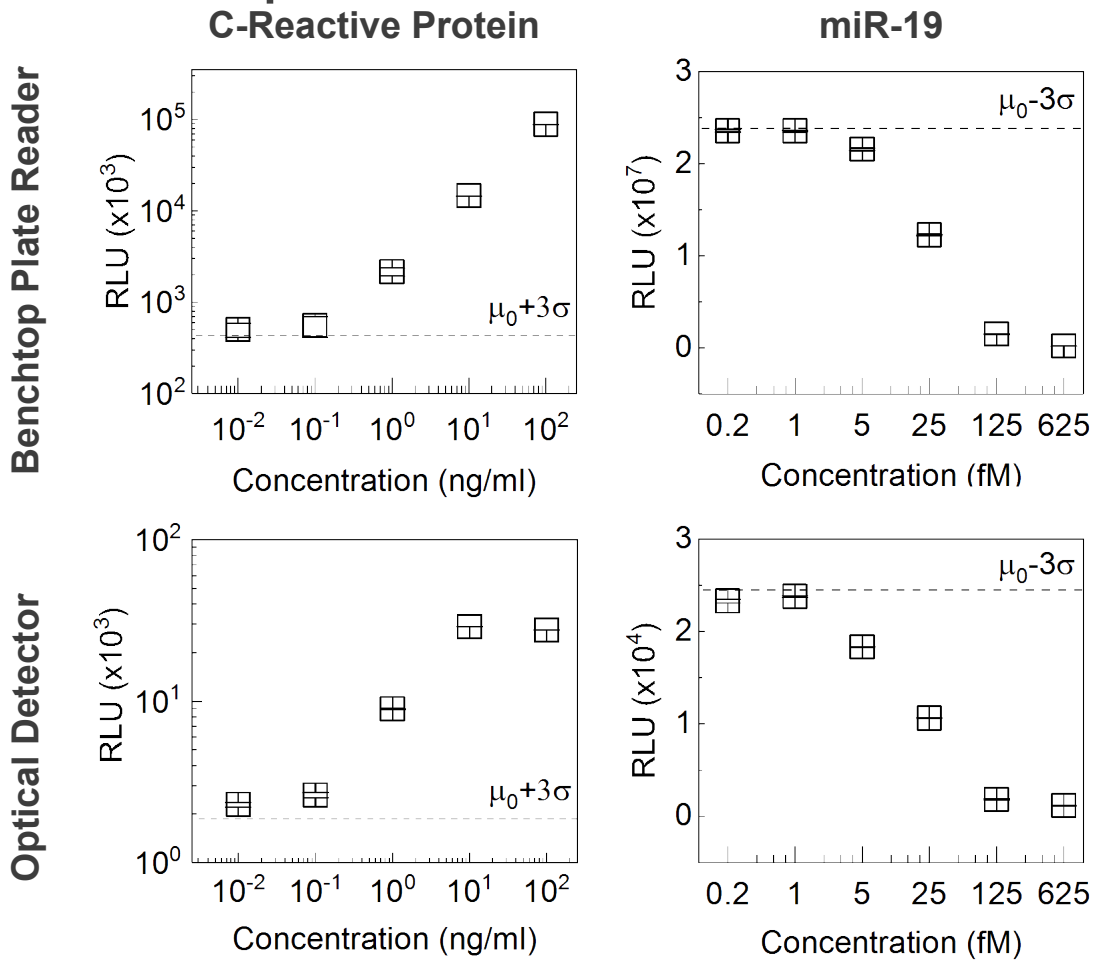
- Excellent uniformity among eight testing units in terms of optical sensing, bead transfer, and on-chip immunoassay tests.
- Robustness of integrated streamlined sample process
- This implies thorough mixing/washing and consistent bead transfer

Protein Biomarker Detection Panel

Target	Description	Function	Normal range in serum	Elevated levels in serum (infected)	Assay sensitivity in serum acceptable?	Detected Normal Human Serum
CRP	C-reactive protein	Acute inflammatory response biomarker for tissue damage or infection, systemic inflammation	<0.3ng/mL	50 ng/mL	YES LoD: 0.1 ng/mL	0.1 ng/mL
IL-6	Interleukin-6	Systemic infection, biomarker for severe pneumonia, predictor of disease progression	<0.05 ng/mL	>0.05 ng/mL	YES LoD: 0.01 ng/mL	0.02 ng/mL
IP-10	IFN-γ-induced protein 10	Biomarker for severity of acute respiratory infection	0.05 ng/mL	1 ng/mL	YES LoD: <0.1 ng/mL	0.1 ng/mL
MxA	Myxovirus Resistance Protein Marker A	Biomarker for acute viral infections	100 ng/mL	400-800 ng/mL	YES LoD: 0.6 ng/mL	98 ng/mL
PCT	Procalcitonin	Indicator of bacterial infection and sepsis	<0.1 ng/mL	>2 ng/mL	YES LoD: 0.5 ng/mL	Not detectable
PTX3	Pentraxin-3	Systemic inflammation indicator	not detectable	71 ng/mL	YES LoD 0.9 ng/mL	In progress
TRAIL	TNF related apoptosis inducing ligand	Elevated in viral infection, inverse correlation to bacterial infection, hypercholesterolemia indicator	90 pg/mL	0.05 ng/mL	YES LoD: 0.9 ng/mL	In progress

- On-going effort on bead-based sandwich immunoassay development to differentiate “virus vs bacterial” infection.

Preliminary Sensitivity Results with Platform Under Development



- Microfluidic bead-based immunoassay and molecular assay achieved detection sensitivity down to ~100 pg/mL for CRP and ~5 fM for miR19 using optical detector integrated in the multimode assay platform.