

Multiplexed Host Response Biomarker Analysis on a Rapid, Quantitative Point-of-Care Platform

Daniel T. Nieuwlandt, Philip Papst, Michael J. Lochhead*, MBio Diagnostics, Inc, Boulder, CO



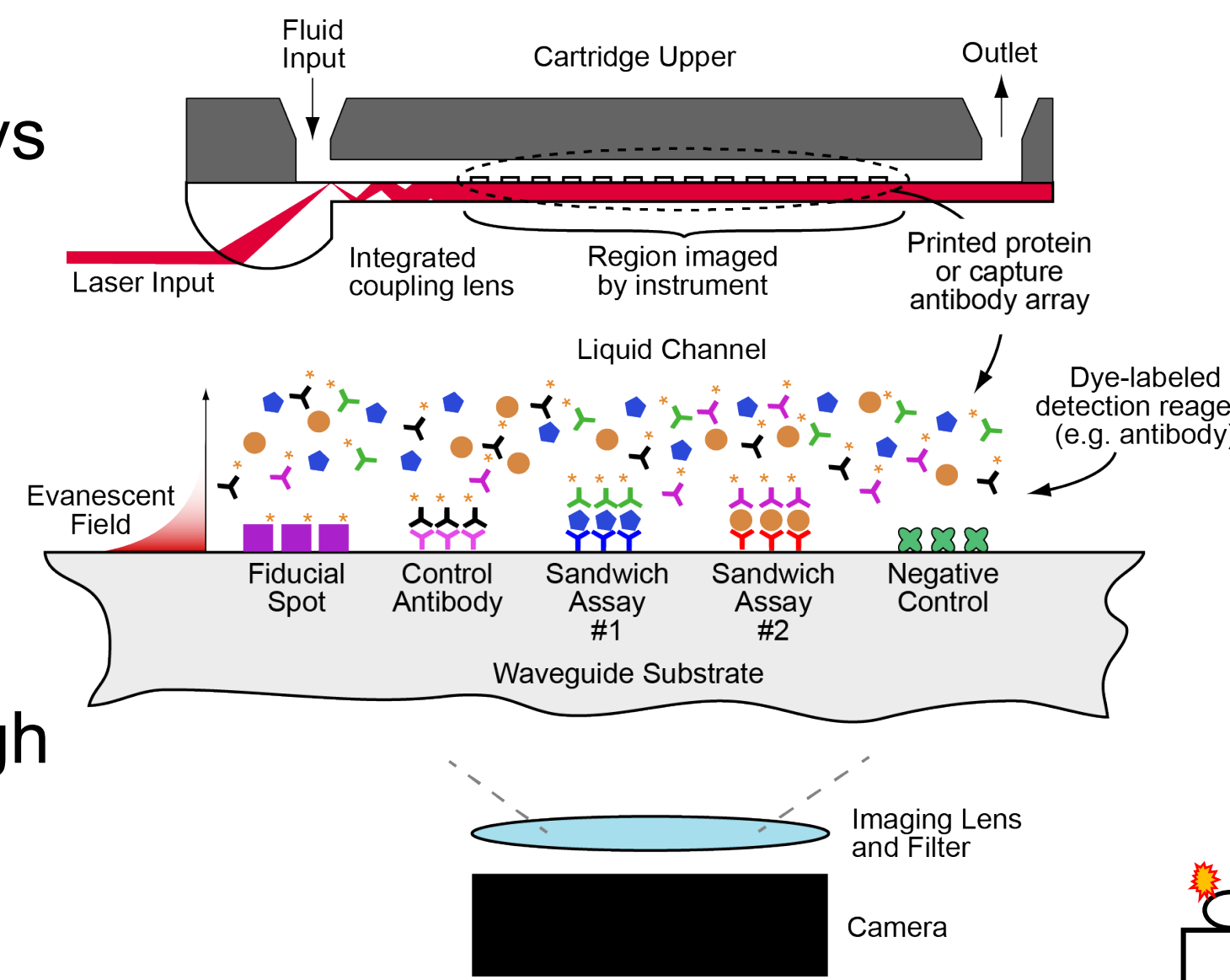
SUMMARY

- Rapid (< 30 min) detection of circulating host-response protein biomarkers could provide actionable diagnostic or prognostic information during temporally complex conditions such as acute infection
- Results are presented demonstrating rapid, multiplexed host response immunoassays using MBio's point-of-care assay platform
- Analytical performance data show simultaneous, quantitative biomarker detection over clinically relevant ranges
- Clinical sample performance data demonstrate quantitative detection in pediatric serum samples from patients clinically classified as sepsis, septic shock, and SIRS
- New data demonstrating a simplified workflow and expansion to a 4-plex assay are provided

Biomarker	Description
Procalcitonin (PCT)	Marker for systemic bacterial infection; increasingly used in US for acute infection therapy management
C-Reactive Protein (CRP)	Inflammation marker associated with acute phase infection (among other indications); commonly ordered during sepsis workup
Interleukin-6 (IL-6)	Inflammation marker associated with acute infection
Inducible Protein 10 (IP-10)	Interferon gamma-induced protein 10 (IP-10 or CXCL10); CXC chemokine under investigation as acute infection marker

MBio LIGHDECK® TECHNOLOGY

- Fluidic cartridge-based multiplexed immunoassays
- Novel planar waveguide-based illumination
- Fluorescence imaging detection
- Spatial multiplexing with microarray technology
- Quantitative output through proprietary software

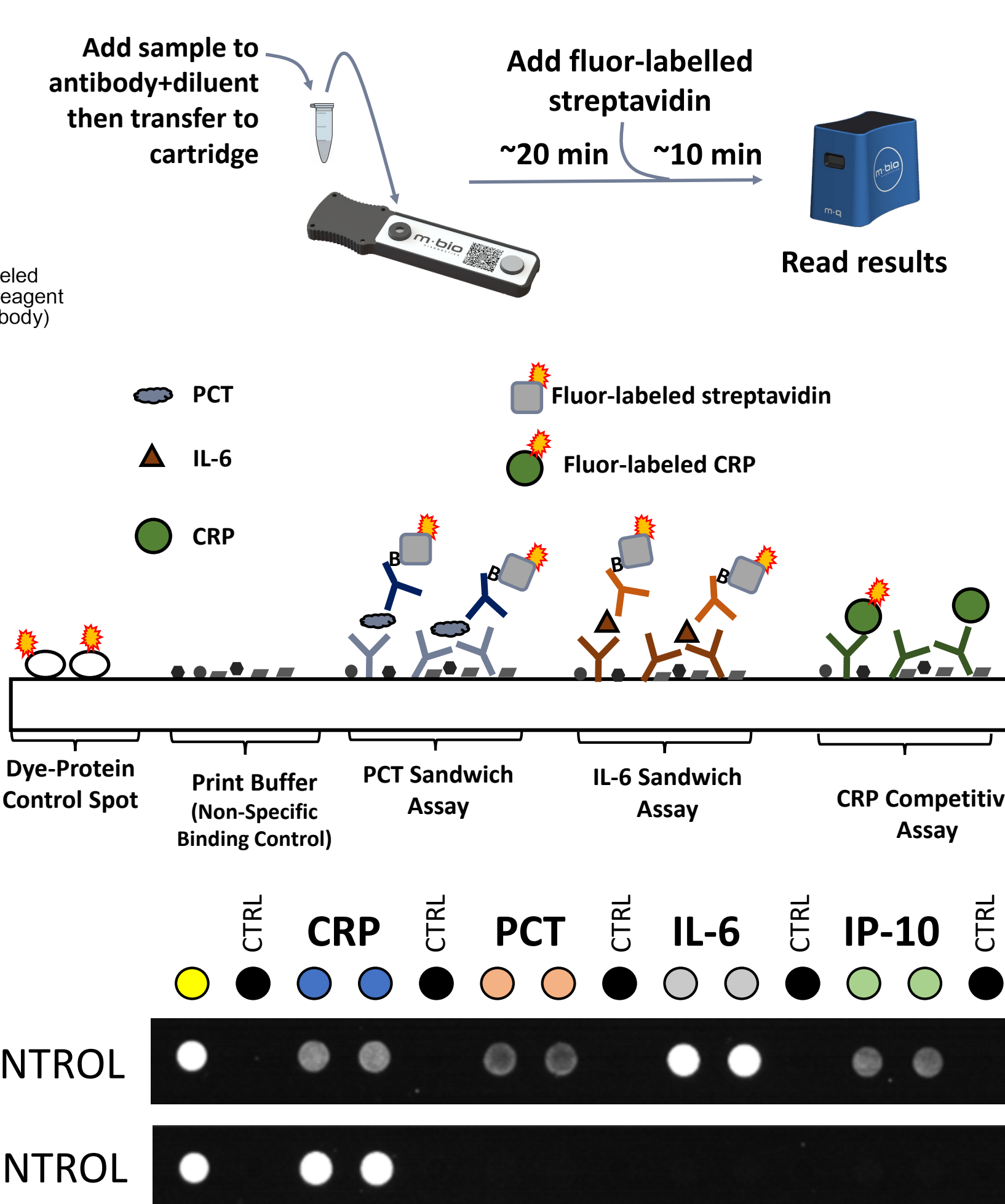


SYSTEM FEATURES:

- Multiplexed** – 6 to 90 (depending on assay)
- Rapid** – 1 to 5 minutes; < 30 minutes
- Quantitative** – fluorescence immunoassay
- Sensitive** – picogram/mL immunoassays
- Simple** – path to CLIA-waivable assays
- Robust** – field-ready technology
- Versatile** – multiple sample matrices demonstrated including serum, plasma, whole blood
- Scalable** – designed for manufacture
- Cost-effective**

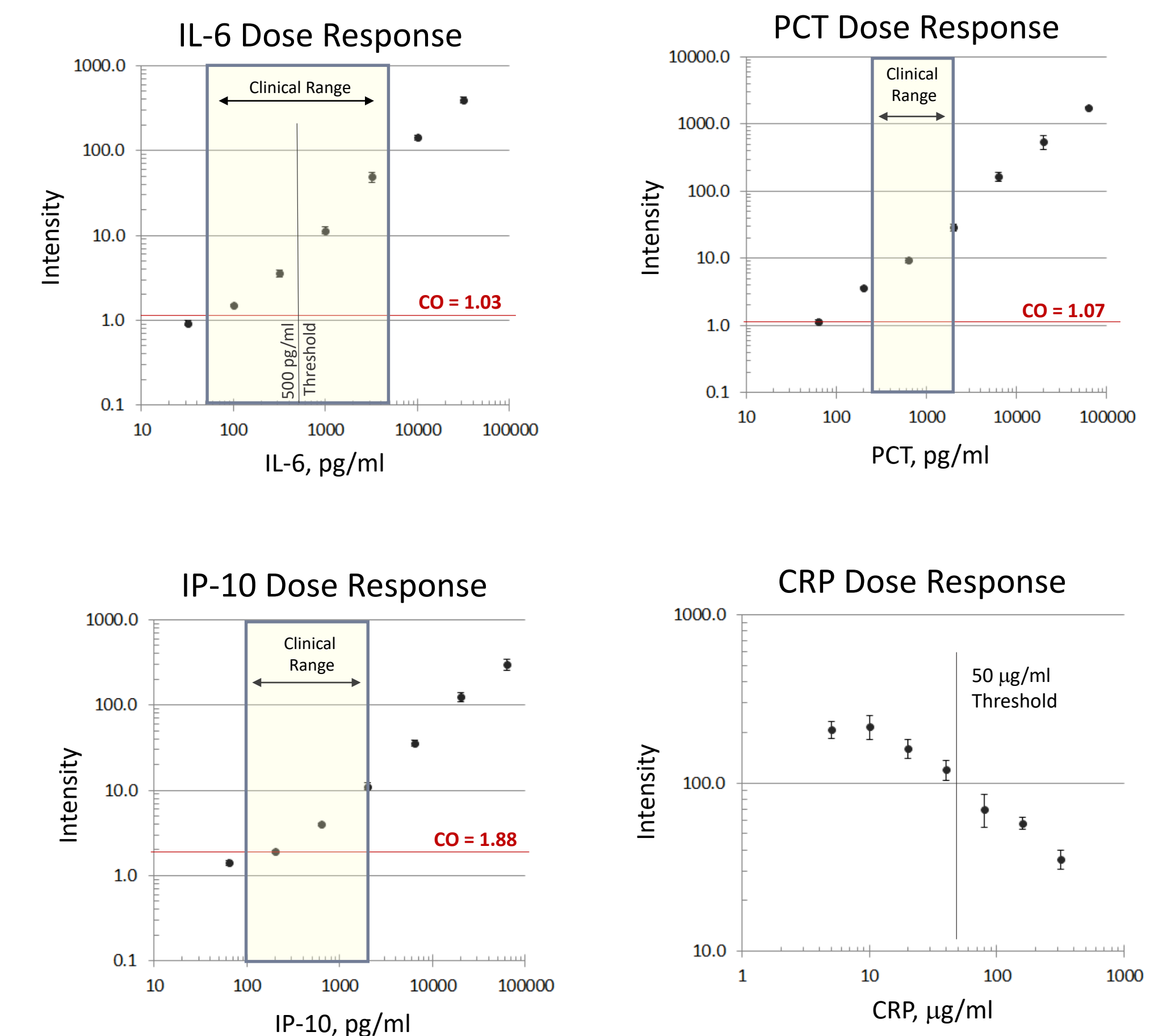
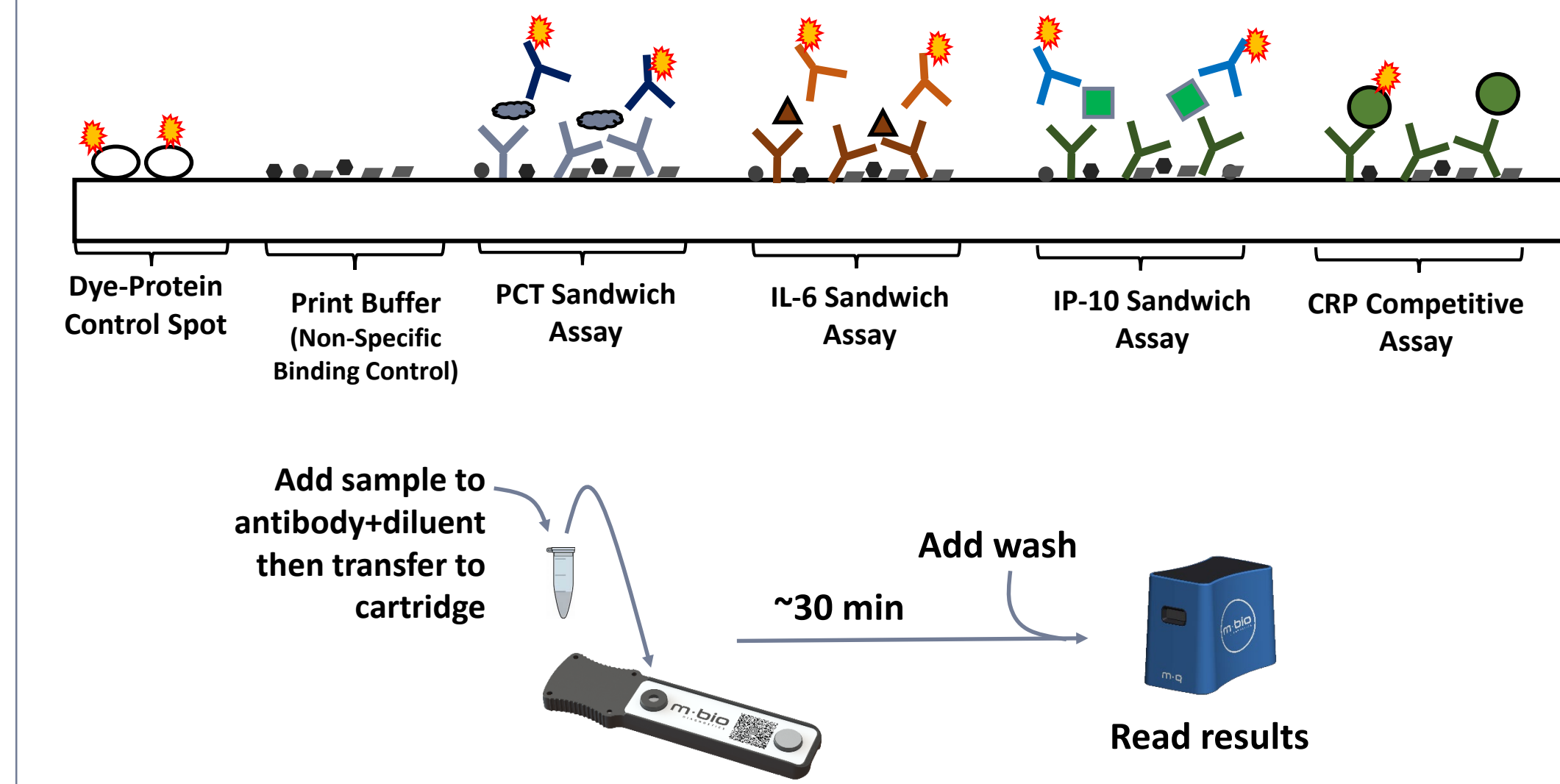
Demonstration Assay Format

Quantitative Tri-Plex Immunoassay with Controls



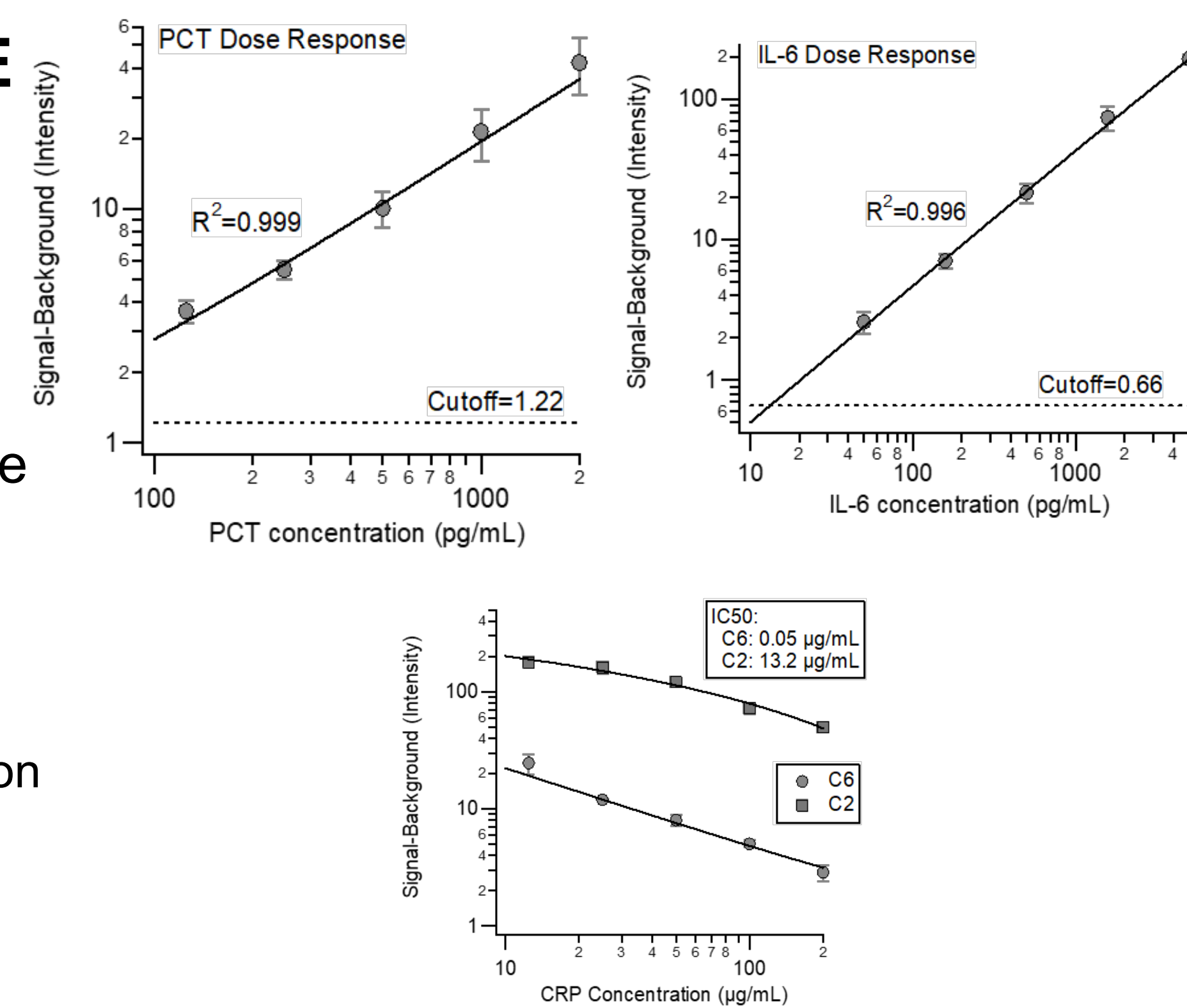
WORKFLOW AND ASSAY IMPROVEMENTS

- Direct dye-conjugation of detection reagents; eliminates an assay step
- Added IP-10 to make a 4-Plex Assay
- Expanded dynamic range of PCT assay



CALIBRATION / ANALYTICAL PERFORMANCE

- Serial dilution of recombinant proteins in PBST use to establish calibration curves
- Each symbol is the average of triplicate cartridges
- Error bars are ± 1 standard deviation
- Four parameter logistic fits illustrated on graphs
- Cutoff is the mean of the no target (zero) samples plus three standard deviations



Quantitative Assay Method

- Calibration curves are set as part of cartridge manufacturing
- Intensity measurements on the reader are converted to concentration in reader software
- Example quantitative clinical sample results below

CLINICAL SAMPLE DEMONSTRATION

50 clinical serum samples sourced from collaborator Dr. Hector Wong of the Cincinnati Children's Hospital Medical Center. Samples are from a pediatric population, clinically classified as:

- 10 Systemic Inflammatory Response Syndrome
- 10 Sepsis
- 30 Septic Shock

Each MBio cartridge delivers 3 quantitative immunoassay results for each sample. Example results for one sample from a patient clinically classified as septic shock (HW-30):

IL-6: 23 pg/mL
PCT: > 5 ng/mL
CRP: 42 µg/mL

MBio data generally correlate with reference ELISAs
Dataset demonstrates performance in relevant clinical samples

IL-6 RESULTS

Clinical Category	ELISA IL-6 (pg/mL)	MBio IL-6 (pg/mL)
SEPSIS	92	94
SEPSIS	118	117
SEPSIS	out of range	1324
SEPSIS	< 3	30
SEPSIS	24	16
SEPSIS	< 3	< 11
SEPSIS	34	69
SEPSIS	10	12
SEPSIS	27	74
SEPSIS	373	< 11
SIRS	< 3	< 11
SIRS	333	192
SIRS	14	< 11
SIRS	15	< 11
SIRS	68	68
SIRS	8	14
SIRS	6	< 11
SIRS	20	140
SIRS	17	< 11
SIRS	41	36

CONCLUSIONS

- Quantitative IL-6, PCT, IP-10, and CRP sandwich immunoassay results were generated on the MBio platform in a 30-minute, cartridge-based assay over clinically relevant ranges
- Overall correlation between MBio and the reference ELISAs
- IL-6 varied with high and low samples in the three clinical categories (sepsis, septic shock, SIRS)
- 29 of 30 samples from septic shock patients showed PCT well above threshold
- Broad dynamic range PCT assay
- The CRP assay showed correlation with ELISA, but there were several sample with significant quantitative differences suggesting the CRP assay needs further optimization

ACKNOWLEDGEMENTS

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