

# MBio Acute Infection Host-Response Quantitative Panel

## SUMMARY

This MBio White Paper provides preliminary research data on a rapid, quantitative, *multiplexed* host-response marker immunoassay run on the MBio LightDeck® point-of-care cartridge and reader platform. Acute infections evolve rapidly in an individual, so providing time-critical information to the clinician can significantly affect patient management. The initial panel includes Interleukin 6 (IL-6), C-reactive Protein (CRP) and procalcitonin (PCT). The dynamic range is limited in this initial data set, while 3-plex multiplexing is demonstrated. We anticipate that host response panels will find use in a variety of indications, and additional markers can readily be added to the MBio cartridge.

## BACKGROUND

The management of patients with acute severe infection, particularly in the context of sepsis and septic shock, remains one of the most vexing problems in medicine. Sepsis is a devastating cause of morbidity and mortality in intensive care units (ICUs), with estimates of U.S. incidence ranging from 894,000 to 3.1 million cases annually. The heterogeneity of clinical presentation (etiology, patient history, severity, etc.) creates a major challenge for patient management and has significantly complicated development of new therapies. Because of its high incidence and high cost of treatment, sepsis diagnosis related groups are the most expensive conditions treated in US hospitals, accounting for 6.2% of all costs in 2013. Sepsis-related diagnoses are the second-leading cause of hospital readmissions. Treatment is complicated by the fact that many of the signs and symptoms are non-specific, and they are common to other non-infectious sources of systemic shock. A panel approach to host-response markers is warranted. In general, targeted therapy for acute infections will be valuable in both sepsis and the general need for antibiotic stewardship. The primary focus of the MBio Host Response Quantitative Panel program is detection of known host response biomarkers in a novel point-of-care multiplexing package within minutes.

## MBIO SYSTEM DESCRIPTION

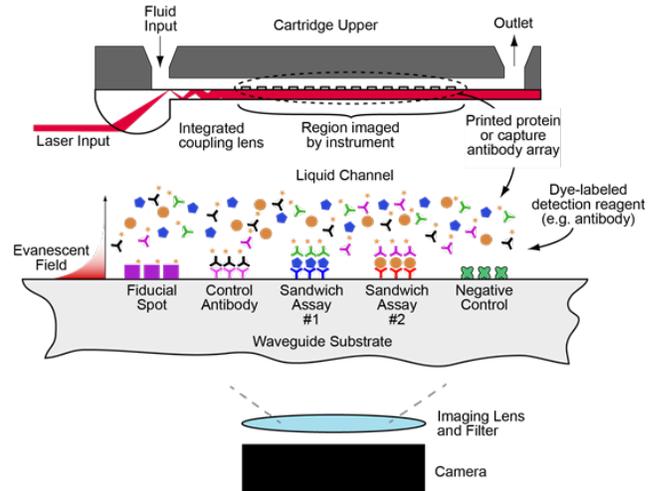
The MBio platform has been developed to deliver quantitative, multiplexed, fluorescence immunoassay results using a simple disposable cartridge and portable reader (Fig. 1).



**Figure 1.** MBio cartridge and readers

The platform is based on a patent-protected optical approach that combines planar waveguide-based illumination, microarray technology, microfluidics, and fluorescence imaging. MBio's

LightDeck® technology enables sensitive fluorescence immunoassay measurements in a simple, low-cost, easy-to-use package. Fig. 2 provides a schematic of the LightDeck® technology components.

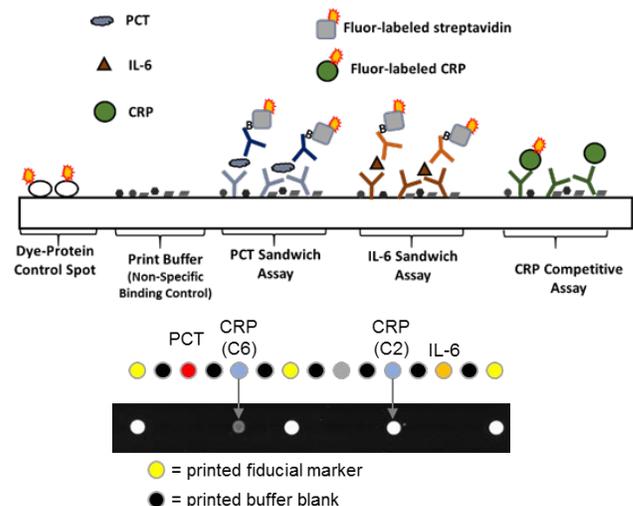


**Figure 2.** MBio LightDeck® technology components.

The technology has been demonstrated for a variety of applications, including human clinical diagnostics<sup>1,2</sup>, blood cell counting<sup>3</sup>, food safety<sup>4</sup>, and environmental monitoring<sup>5-8</sup>.

## HOST-RESPONSE PANEL

The prototype host-response assay described here combines inflammatory markers procalcitonin (PCT), interleukin-6 (IL-6), and C-reactive protein (CRP) immunoassays on a single cartridge. PCT and IL-6 are configured as sandwich immunoassays. CRP is configured as a competitive immunoassay. Fig. 3 provides a schematic of the assay layout in the MBio cartridge and a representative image generated with the MBio reader.



**Figure 3.** PCT/IL-6/CRP assay schematic and representative array image from the MBio reader.

**MATERIALS AND METHODS**

*Materials.* All biological reagents (antibodies, recombinant proteins, streptavidin-dye, etc.) were sourced commercially.

For standard curve development, recombinant proteins (PCT, IL-6, CRP) were used as target analytes. Serial dilution into normal human serum was used to establish assay analytical performance.

*Cartridge Assembly.* Multiplexed immunoassay cartridges were printed and assembled at MBio Diagnostics. Cartridges comprise a microarray of printed capture antibodies, antigen, print buffer and control spots, integrated into the MBio plastic fluidic cartridge.

A detection reagent cocktail comprising biotinylated antibodies and the dye-conjugated CRP detection reagent was lyophilized into the inlet port of the cartridge as part of manufacturing.

*Assay Workflow.* The simple two-step developmental workflow shown in Fig. 4 was used to generate standard curve results.



Figure 4. Assay workflow.

**RESULTS**

Standard curves of the three biomarkers are presented in Figs. 5-7. In each case, three replicates (three cartridges) were run at each concentration. Data are presented as averages of triplicate measurements, with error shown as ± one standard deviation. A 4-parameter logistic fit is shown on each plot.

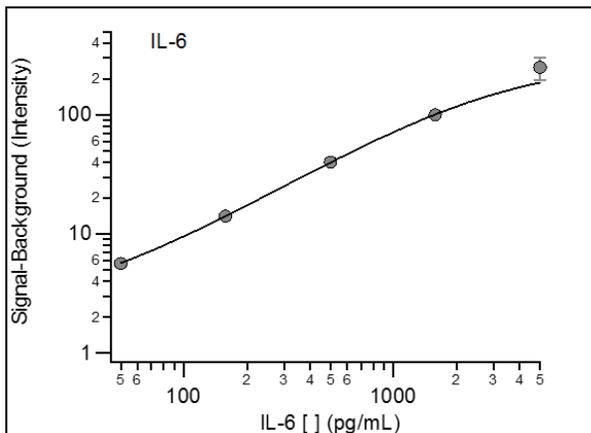


Figure 5. MBio interleukin-6 assay standard curve.

Analytical performance of the IL-6 assay includes: 20.5 pg/<math>mL</math> limit of detection (LOD), ~50 pg/<math>mL</math> limit of quantitation (LOQ), and a range of 25-5000 pg/<math>mL</math>.

PCT data are provided in Fig. 6. PCT analytical performance parameters are LOD: 21.9 pg/<math>mL</math>; LOQ: 50 pg/<math>mL</math>; and Range: 50 – 5000 pg/<math>mL</math>. Analysis of clinical samples suggest over 3 logs of dynamic range.

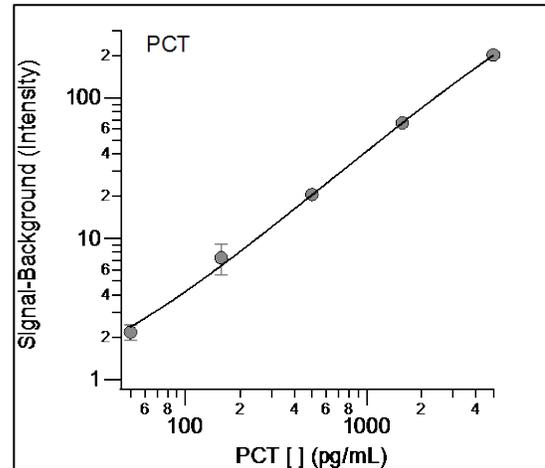


Figure 6. MBio PCT assay standard curve.

CRP is based on a competitive format, so the standard curve is an inhibition curve as shown in Fig. 7. For this assay, we report analytical performance as Low (< 40 μg/<math>mL</math>), Medium 40 – 100 μg/<math>mL</math> (quantitative range), and High (> 100 μg/<math>mL</math>).

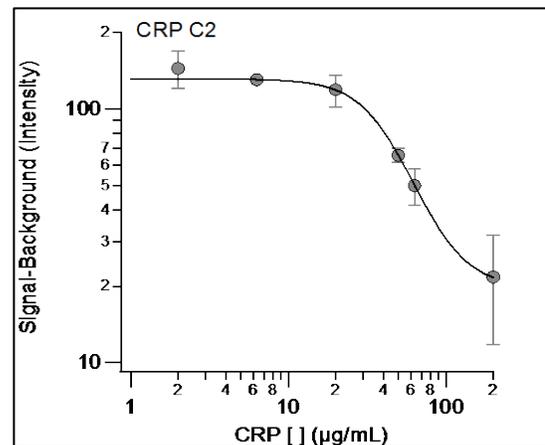


Figure 7. MBio C-Reactive Protein standard curve.

**CONCLUSION**

This brief dataset demonstrates MBio's ability to generate multiplexed, quantitative immunoassay results on a simple, portable cartridge and reader system. The system is extensible to larger panels, lower limits of detection, and wider dynamic range. Further applications will be described in the near future.

**REFERENCES**

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