**MBio Veterinary Testing Applications: Canine CRP**

**SUMMARY**
This MBio Technical Report provides preliminary data on a rapid, quantitative test for canine C-reactive protein (cCRP) via immunoassay run on the MBio LightDeck® point-of-care cartridge and reader platform. cCRP is an acute phase protein produced mainly in the liver in response to inflammatory stimuli, arising out of infection and/or tissue damage. Its concentration rises rapidly during systemic inflammation and decreases quickly after resolution of the inflammatory stimulus. The magnitude of rise can be up to 1000-fold, necessitating a quantitative assay with a robust dynamic range. Canine CRP can be used in diagnosis and monitoring of a variety of conditions, from acute infections, inflammatory bowel disease, acute pancreatitis, and surgical complications.

**BACKGROUND**
As with humans, canine C-reactive protein can be used prognostically and in monitoring response to treatment in dogs. Studies have shown the value of cCRP in a veterinary context, with applications in parvovirus infection, anemia, and pancreatitis. The levels of CRP will rise within a few hours of an inflammatory stimulus, and begin to decline within 24 hours of initiation of treatment. The time response of this marker is faster than other common markers of inflammation, such as sedimentation rate, white blood cell count, SAA, and fibrinogen, making CRP a good marker for treatment response. The range of concentrations of this marker can be large, from less than 5 µg/ml in healthy individuals, rising to as high as 600 µg/ml in severe bacterial infections. Pregnancy also elevates the levels of CRP. Since CRP can be elevated by multiple stimuli, it is an indicator marker that should be used in conjunction with clinical signs and symptoms for diagnostic purposes.

**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](image)

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.](image)

The technology has been demonstrated for a variety of applications, including human clinical diagnostics, food safety, and environmental monitoring. CANINE C-REACTIVE PROTEIN
Canine C-reactive protein is a large (~115kDa), glycosylated pentamer. In contrast, human CRP is non-glycosylated. Monoclonal antibodies (mAb) specific for canine CRP have been developed, and pairs of antibodies can be configured as a sandwich immunoassay. Due to the very high concentration of CRP relative to other signal proteins, a dilution step is common for quantitative CRP assays, and this approach is used here. The C-reactive protein assay described here is a sandwich immunoassay with positive and negative controls, plus signal calibrators all on-board a single disposable plastic cartridge. Fig. 3 provides a schematic of the assay layout in the MBio cartridge and a representative image generated with the MBio reader.

![Figure 3. CRP assay schematic for a sandwich assay. Note that the assay requires no wash due to the selective surface illumination provided by the waveguide technique.](image)
**RESULTS**

A standard curve for the cCRP assay is presented in Fig. 5. For each cCRP concentration data point, three replicate assays (three cartridges) were performed. Data are plotted as averages of these triplicate measurements, with error bars representing ± one standard deviation. The error bars are present, but are not visible on the log plot. Reproducibility for the triplicate measurements was ~ 7%CV across the assay range.

A 4-parameter logistic (4PL) fit of the data is shown on the plot. Lower limit of detection (LLOD) is defined as the minimum concentration detectable above the zero analyte samples. We statistically define LLOD as the mean of the “zero analyte” replicates plus 3 standard deviations. Using six replicates of the zero analyte control, and correcting for the 2000x dilution, LLOD is 0.8 µg/mL. Assay quantitative range is 1 to 4,000 µg/mL. This three-log range can easily be shifted to lower or higher concentrations by changing the specimen dilution factor.

**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, wider dynamic range and on-board dried assay reagents permitting single step addition of diluted serum specimens to the MBio cartridge. Further applications will be described in the near future.

**REFERENCES**

10. S. E. McNamee, et. al., Environmental science & technology, 2014;48, 13340-13349.