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Myriad RBM, Inc. is a CLIA-certified biomarker testing laboratory that solves complex drug development and diagnostic challenges with innovative products and services. With more than a decade of experience developing and validating multiplexed immunoassays, we provide services for the identification and quantification of important biomarkers.

Our Multi-Analyte Profile (MAP) services can quantitatively measure hundreds of proteins from just a single drop of blood serum or plasma. The ability to provide accurate data on this scale, from such small sample volumes, is made possible thanks to our expertise in multiplexed immunoassay design and development combined with the automated improvements implemented to optimize the Luminex technology.

Briefly, samples are electronically logged, manually plated alongside controls, then run on a robotic system that performs all of the volume critical steps including dilutions, standard curves, and combination of reagents, before each sample is read on a Luminex instrument (more detailed information can be found in our QC white paper). The automation and rigorous application of these methods distinguishes our services in terms of precision and efficiency.
Background and Myriad RBM Cardiovascular Services

Cardiovascular disease (CVD) is the leading cause of mortality worldwide with an estimated 17.3 million deaths from CVD in 2008. Of these deaths, 7.3 million were due to coronary heart disease and 6.2 million were due to stroke. It is estimated that over 23 million people will die from CVD in the year 2030, and that it will likely remain the leading cause of death for the foreseeable future. Statistics such as these clearly demonstrate the need for an intense focus on developing and improving tools used in the diagnosis and treatment of CVD. One approach where significant headway has been made is through the use of biomarkers. Biomarkers are powerful tools for diagnosis in the earliest stages of disease and effective in later stages by monitoring treatment efficacy and post-treatment complications. In many cases, the use of novel biomarkers in addition to conventional cardiovascular risk predictors (i.e. cholesterol, blood pressure, etc.) enhances the information available to clinicians and increases specificity and sensitivity in disease detection.

Our biomarker panels are referenced in nearly 50 publications demonstrating the utility of the multiplexed immunoassay approach across a range of studies involving all stages of CVD from diagnosis to prognosis and treatment evaluation.

Atherosclerosis

Cardiovascular disease and morbidity rates increase substantially with age, yet our understanding of the mechanisms underlying cardiac aging and disease is limited. A number of recent clinical studies, however, have illustrated the power of multianalyte profiling as a tool for tracking CVD progression. One example, Tantry et al., outlined here focuses on coronary artery disease (CAD), the narrowing or blockage of the coronary arteries, typically by atherosclerosis. Myriad RBM’s services were used to demonstrate a significant correlation between the expression of coagulation and inflammation markers in the progression of CAD from asymptomatic (AS) to symptomatic stable angina (SA) or unstable angina (UA). Coagulation markers such as von Willebrand factor, showed significant increases between the UA group and both SA and AS groups. Inflammation markers including C-reactive protein, RANTES, IL-8, IL-1α, IL-4, IL-7, IL-10, MIP-α, MIP-β and VCAM-1 all showed significant increases between AS and SA and AS and UA groups. The ability to simultaneously measure the expression of such a large number of markers at various stages of disease progression may improve the identification of patients at risk for an ischemic event, allowing for more effective therapeutic intervention.

Figure 1: Example of biomarker profile for two coagulation markers in the different stages of coronary artery disease acuity.
Another study utilizing Myriad RBM’s services demonstrated that enhanced expression of coagulation and inflammation markers, when correlated with heightened thrombogenicity, could help predict the likelihood of a recurrent ischemic event following stenting\(^6\). Eighty-four patients were grouped into quartiles based on measurements of the tensile strength of thrombin-induced platelet-fibrin clots (MA). Patients with high-MA (4th quartile) showed elevated levels of fibrinogen and von Willebrand factor as well as C-reactive protein and IL-8 compared to low-MA patients (1st quartile). Furthermore, nearly 50% of patients with high-MA experienced an ischemic event within 2 years, whereas only 13% of low-MA patients experienced an ischemic event in the same timeframe. This study is a first step towards developing a biomarker profile to facilitate the assessment of post-stenting ischemia risk and its use could lead to personalized antithrombotic strategies.

**Figure 2:** Quantification of various biomarker levels relative to quartiles of platelet-fibrin clot strength (MA). p-values shown are for comparison between each quartile and the 4th quartile. The bottom right panel shows Kaplan-Meier curves of cumulative first ischemic event occurrence in patients grouped by MA quartiles. p-values are again shown for comparison to the 4th MA quartile.
Risk of Complication After Cardiopulmonary Bypass Procedures

One final example of the application of our MAPs is in the identification of biomarkers used to assess patient complication risk following cardiopulmonary bypass procedures (CPB) during pediatric heart surgery. Agirbasli et al. screened plasma samples from patients undergoing non-pulsatile CPB at various timepoints before, during, and up to 24 hours post procedure. As only 100μL of plasma are required at each time point, this type of assay is particularly well suited to pediatric studies where patients often cannot tolerate large plasma sample collection. Using Myriad RBM’s services to test the expression levels of 90 biomarkers at various time points, investigators saw significant changes in markers of inflammation, chemokines, lipoproteins and hormones, as well as indicators of myocardial injury.

Of note, the authors found three novel biomarkers to be early indicators of myocardial injury including most significantly, heart-type fatty acid-binding protein (FABP). These markers were the first to rise, faster than traditional markers of injury, and were detectable at levels between 18 and 50 times greater than baseline levels in just 3-5 minutes after CPB intervention (see Fig 3). The authors also found that the expression of inflammatory biomarkers such as IL-6 and IL-10 can be used not only to assess the onset of the inflammatory response in the hours immediately following CPB, but also might serve as a predictor of patient outcome when monitored 24 hours post-surgery.

Successfully identifying the expression levels of various cardiovascular biomarkers in relation to specific patient outcomes will be instrumental in predicting, diagnosing, and treating post-operative complications that arise following CPB in the pediatric population.

Figure 3: Schematic display of all biomarkers demonstrating the early increase of myocardial injury markers followed by increases in various inflammation markers. The y-axis is the fold-increase in expression level as compared to the pre-CPB value.
Conclusion

The studies highlighted here demonstrate the unique advantages of using Myriad RBM’s powerful multiplexed immunoassays to accelerate research on cardiovascular diseases. From enhancing our understanding of the mechanisms underlying diseases and early diagnosis to monitoring efficacy and the development of complications following specific disease interventions, biomarker profiling proves to be an invaluable resource. Myriad RBM strives to be a leading provider of biomarker assays for cardiovascular disorders.
