**Results**

*Validation of Bead-based Specificity Assay*

The first step toward developing a new specificity assay involved validating a bead-based construct and the standard SMP poly-specificity reagent. Protein A functionalized beads were chosen due to their simplicity and yet high efficiency capture of antibodies in an oriented manner that would display the complementarity determining regions (CDRs) outward. This was considered beneficial due to the assumption that the CDRs play a large role in determining specificity (Rabia et al. 2018, Raybould et al. 2019, etc.) Incubation of the beads with a range of antibody concentrations surrounding the estimated bead binding capacity demonstrated a clear dose-response curve (Figure 2). This indicates sensitivity of the assay to the quantity of antibody present. At approximately three times the reported binding capacity of the beads, the measured signal plateaus, indicating saturation of the beads and demonstrating reproducibility of the assay for antibody concentrations above that threshold. With the performed volumes, results were reproducible with antibody concentrations as low as 4.6 μg/mL, however, 15 μg/mL was used for all subsequent analyses to guard against experimental error contributing significantly to conclusions and still requiring less than 2 μg of protein per sample.

*Evaluation of Reagent Concentration Sensitivity*

To evaluate the sensitivity of the assay to reagent concentrations, three dilutions of SMP reagent were analyzed for a panel of five control antibodies (Figure 3). Consistent trends were observed across all concentrations of SMP indicating robustness of the assay against moderate alterations of the reagent conditions. This also indicates the potential to obtain reproducible results with SMP concentrations as low as 0.03 mg/mL.

*Evaluation of Additional Specificity Reagents*

To expand upon the assay developed, additional diagnostic molecules were then evaluated as potential specificity reagents. These new molecules represented a wide variety of molecular properties to probe the nature of specificity as a biophysical property and the diagnostic capabilities of the standard specificity assay. These reagents also represented less expensive and more simple alternatives to cellular protein fractions which are complicated and costly to produce. In total, ovalbumin, human serum albumin, ribonuclease A, insulin, chondroitin sulfates, and galactocerebrosides were evaluated (Figure 4, Supplementary Figure 1). Both ovalbumin and human serum albumin showed promising results with high rank order correlations and classification accuracies with respect to the standard assay. Ovalbumin showed the highest correlations and classification accuracy among all reagent tested