

## Suspension Phase Microsphere Immunoassay of Bevacizumab Levels in Ocular Fluids and Sera

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### PURPOSE

Bevacizumab, a humanized monoclonal antibody targeting VEGF, is currently injected into vitreous humor to treat age-related macular degeneration, proliferative diabetic retinopathy and other conditions associated with vascular leakage and angiogenesis. We developed a suspension phase microsphere immunoassay of free bevacizumab levels in ocular fluids and serum to enable pharmacokinetic studies.

### METHODS

The four-layer sandwich immunoassay uses readily available commercial reagents and employs a dual laser flow cytometer (Luminex 100) to record results. Anti-VEGF microspheres, VEGF, bevacizumab, and antihuman immunoglobulin R-phycoerythrin are the four component layers of the immunoassay, respectively. Vitreous levels of bevacizumab are determined without specimen dilution. Sera are diluted 100 fold to decrease inhibition from human immunoglobulins.

### RESULTS

Our assay has a dynamic range of analysis from 5 million pg/ml to 782 pg/ml, the lower limit of detection in both undiluted vitreous humor and buffer. This covers greater than three logs of concentration. We have equivalent dynamic range in a 1:100 dilution of serum. Our limit of detection in serum is 78.2 ng/ml. This assay can be used to determine free bevacizumab levels in vitreous, aqueous or serum in about 3 hours at a reagent cost of \$450 USD for 80 results.

### CONCLUSION

This suspension phase microsphere immunoassay provides stable reagents with good precision, and a broad dynamic range over clinically relevant concentrations. This assay format should enable correlation of clinical findings with measured levels of the therapeutic agent in prospective studies and in analysis of ocular fluids removed at surgery.