Prior to running the Bio-Plex assay, the vitreous samples were thawed, the Bio-Plex machine was calibrated, and the machine’s lasers were warmed for four hours. The standards were reconstituted with 781µL of Standard diluent, and the controls were reconstituted with 250µL of Standard diluent. After vortexing the bottles for 5 seconds, they were iced for 30 minutes. During this 30-minute icing period, the 6.5µm magnetic beads were prepared for the assay. The beads were vortexed for 30 seconds in a foil-covered vial, and then 5,472µL of Assay Buffer and 288µL of beads were added to a 15mL tube, and each well was filled with 50µL of this solution and shaken. After the 30-minute icing period, the samples were diluted with a 4-fold standard dilution series and 50µL of standards, blanks, controls, and samples were added to each well in accordance with the plate layout. This plate was then covered with adhesive plate sealer and foil and was shaken while incubating for one hour. Ten minutes prior to the completion of this incubation, the detection antibodies were vortexed for 5 seconds and 145µL of detection Assay Buffer was added along with 2755µL of Assay Buffer diluent. Once the one hour incubation was complete and the plate was washed three times, 25µL of this diluted detection antibody solution was added to each well and the plate was once again covered and shaken while incubating for 30 minutes.

The Stretavidin-PE (SA-PE) stock solution was prepared 10 minutes prior to the completion of this incubation period. The SA-PE solution was covered with foil and vortexed for 5 seconds prior to adding 60µL of SA-PE as well as 5940µL of Assay Buffer to a separate vial. After the incubation period, the plate was washed with antibodies three times and 50µL of the diluted, vortexed SA-PE solution was added to
each well. The plate was once again incubated for 10 minutes then washed three times with antibodies. The magnetic beads were re-suspended in 125µL of Assay Buffer and added to each well of the plate, which was then shaken for 30 seconds. After removing the plate sealer, the Bio-Plex Machine then read the plate to record measurements for each of the 34 biomarkers.

The VEGF-R3 protein biomarker was analyzed by the eBioscience Ready-Set-Go! (r) ELISPOT kit, using the aseptic protocol. The Functional Grade purified capture antibody was diluted in ELISPOT Coating Buffer and incubated overnight at 4 degrees Celsius, 100 uL/well in an ELISPOT plate. The plate was aspirated and washed twice with 200 uL/well of sterile ELISPOT Coating Buffer and the buffer was decanted after each wash. The plate was blocked with 200 uL/well of complete RPMI-1640 at room temperature for 1 hour and then aspirated. The sample was thawed and prepared along with controls and diluted with RPMI-1640 medium at a density of 2x10^6/mL. The samples were distributed at 100 uL/well and incubated at 37 degrees Celsius, 5% CO2 humidified incubator for 36 hours.

<table>
<thead>
<tr>
<th>Targets</th>
<th>Assay Sensitivity, pg/ml</th>
<th>Assay Working Range, pg/ml</th>
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<tr>
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<td>LOD</td>
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LOD: Limit of Detection, LLOQ: Lower Limit of Quantification, ULOQ: Upper Limit of Quantification