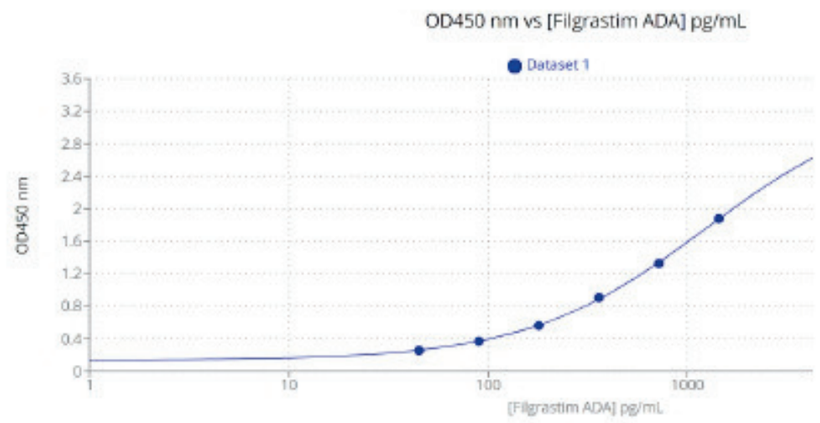
GCSF ADA ELISA
Catalog EL-141-73196

For the determination antibodies to GCSF in serum and plasma.

Introduction

Filgrastim is a granulocyte colony-stimulating factor (G-CSF) analog used to stimulate the proliferation and differentiation of granulocytes. In various clinical trials, filgrastim has been reported to generate an antibody response with both binding and neutralizing antibodies against filgrastim being reported. The formation of binding or neutralizing antibodies to a therapeutic agent may decrease the efficacy of these agents leading to a loss of clinical response over time. In some cases, anti-drug antibodies may cause infusion reactions and serious anaphylactic reactions. Thus, detection, measurement and characterization of anti-therapeutic antibodies are critical in understanding the safety, exposure, and efficacy profile of the therapeutic agent.

Calibration Curve

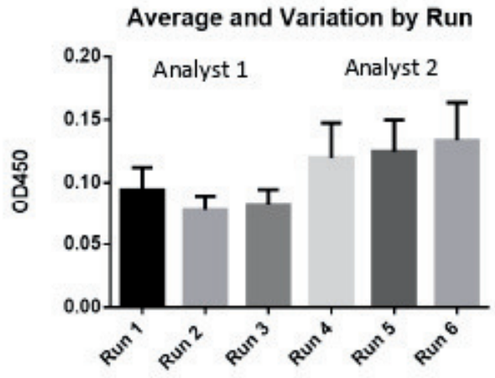


Minimum Required Dilution

Minimum required dilutions of ten (10) unique naïve sera of 1/10, 1/25, 1/50 and 1/100 gave ODs that were below the low-quality control (LQ 100 ng/mL). MRD of 1/50 was used for the remaining validation activities.

Cut Point Determination

Twenty (20) treatment naive samples were tested in duplicate three times by two separate analysts for a total of six experiments. Based on the results of the one-way ANOVA analysis, it was determined that a floating cut point should be used because the source of the differences in means and variances is primarily due to analyst.



Confirmatory Assay

Concentrations of filgrastim above 1000 ng/mL reduced the measurable concentration of anti-filgrastim antibodies (ADAs) in the HQC (800 ng/mL) by half. There was no concentration of filgrastim that reduces the measurable concentration of ADAs to below the detection point.

Lipaemic and Haemolyzed Samples

The haemolyzed sample spiked at the LQ level had % error of ± 25% and the haemolyzed sample spiked at the LQ level in the presence of filgrastim at 120 ng/mL showed a decrease in measured concentration. The lipaemic sample spiked at the LQ level had % error of ± 25% and the lipaemic sample spiked at the LQ level in the presence of filgrastim at 120 ng/mL showed a decrease in measured concentration. No anti-filgrastim antibodies were detected in blank samples of haemolyzed or lipaemic sera.

Specificity

The actual concentrations determined for the high-quality control and low-quality control had % errors ± 25% in the presence of the potential interfering materials (M-CSF, GM-CSF, rheumatoid factor, IL-3 and IL-3RB) and therefore these molecules do not interfere with the assay's ability to measure filgrastim ADAs.

Precision and Sensitivity

The % error for high, medium, and low positive controls was ± 20% for all six runs (three runs by two analysts). The calculated concentrations of anti-filgrastim antibodies for the low, medium, and high positive controls decreased in the presence of filgrastim (120 ng/mL) for all six runs. The %CV for high, medium, and low positive controls was <20% in the absence and presence of filgrastim (120 ng/mL) across six (6) for both analysts.

Freeze/Thaw of Standards

The recovery of the standards prepared with the thawed standard were between 80 – 120% of the theoretical concentration for all standards.

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