Determination of Rituximab in Human Serum by a Gyrolab Assay  
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INTRODUCTION

Rituximab was the first monoclonal antibody approved as a drug for clinical use. The drug targets CD20 on B-cell surface and has been successfully used to treat diseases and disorders that are characterized by having too many B cells, such as a solid tumor of lymphoid cells and rheumatoid arthritis. ELISA is the most common immunoassay platform used for pharmacokinetic analysis of such macromolecules. However, ELISA assays are considered more labor intensive, consume significant amounts of critical reagents, and usually have a narrow dynamic range. New assay platforms with miniaturization and automation such as Gyrolab® are always desirable for quick turn around and ‘fit-for-purpose’ assay development in the CRO environment. Here we report the development and validation of a Gyrolab assay to determine rituximab levels in human serum. The rituximab assay used a sandwich immunoassay format on a BioAssay C2000, in which the analyte is immobilized by a biotinylated monoclonal antibody and is detected by an Alexa-labeled anti-human IgG antibody. The dynamic range of the assay was established to be 90 – 60,000 ng/mL, in human serum. Assay selectivity was evaluated and found to be acceptable for spiked serum samples of both healthy individuals and solid tumor patients. The method was fully validated according to the current industry standards for immunoassays. This is part of our continued effort to implement automation in ligand-binding assays for large molecule bioanalysis at QPS, LLC.

EXPERIMENTAL

Gyrolab Bioassay CD contains streptavidin-bead packed microstructures. Rituximab is captured on the CD200 by biotinylated rat anti-idiotypic monoclonal antibody against rituximab and detected by an Alexa-labeled anti-human IgG antibody (Fig. 1). The rituximab-capture antibody was biotinylated using Sulfo-NHS-LC-Biotin kit and the detection antibody was labeled using Alexa Fluor® 488 Mono-clonal Antibody Labeling Kit. The concentrations of the capture reagent and the detection reagent and the buffer system were optimized to provide a high signal to background ratio for the entire method validation study.

Standards and validation samples were prepared in pooled human serum. All samples were diluted 15 fold in dilution buffer before mixed with Rnxp H-max at 1:1 ratio. The overall minimum required dilution was MRD=30.

RESULTS

The validation of the Gyrolab rituximab assay is summarized in Table 1. Fig. 2 shows typical calibration standards of rituximab in human serum and a 5-parameter logistic fit from 30 to 180,000 ng/mL. Table 2 lists the back-calculated standard concentrations and inter-batch statistical analysis.

Data acquisition at 1% PMT level was used throughout the validation. Regression was performed by Gyrolab Evaluator (v 3.1.5.137, Gyros AB, Sweden) with 5-parameter logistic fit without calibration standards of rituximab in human serum and a 5-parameter logistic fit from 30 to 180,000 ng/mL. Table 2 lists the back-calculated standard concentrations and inter-batch statistical analysis.

In comparison, ELISA assay was conducted using the rat anti-idiotypic monoclonal antibody against rituximab as the coating reagent. The assay plate was incubated with HRP-labeled goat anti-human IgG antibody before adding HRP substrate TMB.

CONCLUSIONS

A Gyrolab method has been validated for the quantitation of rituximab in human serum from 90 to 60,000 ng/mL. With a validated dilution factor of 1000, this assay is suitable for measuring rituximab in human serum from 90 ng/mL to 60,000 ng/mL. The Gyrolab assay was proved to be accurate and selective, with a comparable sensitivity as the ELISA method, but provides a significantly wider assay dynamic range for determination of rituximab in human serum.