Validation of Rituximab Innovator and Rituximab Biosimilar in Human Serum

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INTRODUCTION

Rituximab is a chimeric anti-CD20 monoclonal antibody that is used to treat autoimmune diseases, such as rheumatoid arthritis, and hematological neoplastic diseases. We report here an ELISA method for the quantitative determination of both rituximab innovator and rituximab biosimilar in human serum from normal and targeted populations.

MATERIALS AND METHODS

The rituximab ELISA was constructed as a sandwich assay where rituximab was captured by anti-rituximab antibody coated micro-titer plate and detected by goat anti-human IgG HRP conjugate. Signal generated in substrate is proportional to the concentration of the rituximab concentration in samples. Assay was first developed in normal human serum and then optimized for the analysis of rituximab in serum from solid tumor and rheumatoid arthritis (RA) patients.

The innovator was used as reference material. The similarity between two sources of the innovator, i.e. EU and US, was confirmed in this assay and the similarity in the analysis of the innovator (EU) and the biosimilar was demonstrated. Respective validation samples were prepared with rituximab innovator and biosimilar respectively, and analyzed against calibration standards made from the reference material. The following parameters were established in the validation.

- Intra-assay and inter-assay precision and accuracy
- Selectivity in normal ,solid tumor and RA patients' serum samples
- Selectivity in hemolyzed and hyper-lipemic serum samples
- Specificity of the assay with the presence of anti-drug antibodies
- Dilution linearity
- Stability: Room temperature and freeze/thaw stability of both innovator and biosimilar

VALIDATION RESULTS

The intra- and inter- assay precision and accuracy was determined from six runs performed over at least 3 days by more than two analysts. The total error was calculated from all accepted runs (Table 1).

Table 1. Intra-Assay and Inter-Assay Precision

	Comparison of Rituximab Innovator EU and US		Comparison of Rituximab Innovator and Biosimilar	
	Rituximab-EU	Rituximab-US	Rituximab-EU	Rituximab-Pfizer
Intra-Run Precision (%CV)	4.9% to 9.0%	2.9 to 9.4%	6.4 to 13.8%	4.5 to 9.8 %
Intra-Run Accuracy (%RE)	-8.8% to -1.6%	-5.3 to 5.7%%	-10.3 to 2.0%	-16.0 to -9.0%
Inter-Run Precision (%CV)	5.3% to 9.5%	4.1 to 10.7%	7.3 to 13.8%	5.4 to 11.9%
Inter-Run Accuracy (%RE)	-8.8% to -1.6%	-5.3 to 5.7%	-10.3 to 2.0%	-16.3 to -9.0%
Total Error	9.3 to 15.8%	13.2 to 19.6%	13.2 to 19.6%	14.4 to 23.5%

Matrix selectivity (recovery) was demonstrated by the analysis of individual lots of serum, neat or spiked with analytes at LQC and HQC concentration levels. The specificity were found acceptable for Rituximab-EU, Rituximab-US and Rituximab-Pfizer in 10 lots of normal, 10 lots of solid tumor and 20 lots of RA patient serum samples (Table 2A).

Table 2A. Matrix Selectivity (% Recovery) of Rituximab in Human Serum

		Rituximab-EU		Rituximab-US		Rituximab-Pfizer	
Normal Lot	Blank	LQC	HQC	LQC	HQC	LQC	HQC
BRH513581	BLQ	93.3	97.3	96.7	92.0	108.3	92.5
BRH513582	BLQ	83.3	90.8	90.3	93.5	94.7	86.3
BRH513583	BLQ	98.7	106.5	106.0	99.3	113.3	92.0
BRH513584	BLQ	104.0	106.8	94.0	88.3	94.3	77.5
BRH513585	BLQ	96.3	101.3	96.3	95.0	98.3	84.0
BRH513586	BLQ	91.7	92.0	90.3	89.8	100.7	89.0
BRH513587	BLQ	119.3	95.5	113.3	102.8	112.0	83.5
BRH513588	BLQ	94.3	104.0	86.3	86.3	97.3	88.8
BRH513589	BLQ	88.7	92.5	97.7	95.5	96.7	79.0
BRH513590	BLQ	99.0	98.5	100.3	100.8	101.3	91.5
Percentage of lots met the criteria		100.0%		100.0%		80.0%	

Solid Tumor	Blank	LQC	HQC	LQC	HQC	LQC	HQC
BRH502734	BLQ	115.7	102.5	115.0	87.0	111.0	97.5
BRH502735	BLQ	101.3	95.0	101.7	96.3	107.0	102.3
BRH502736	BLQ	90.3	100.0	88.3	89.0	95.3	108.3
BRH502737	BLQ	96.0	99.8	85.7	81.3	97.0	94.0
BRH502738	BLQ	106.0	110.3	92.7	86.5	102.3	103.5
BRH502677	BLQ	96.0	98.5	89.0	83.5	92.3	95.0
BRH502678	BLQ	92.7	103.3	88.3	85.5	94.3	94.3
BRH502679	BLQ	98.0	103.3	87.3	81.8	98.0	98.0
BC20-111	BLQ	92.0	97.8	94.3	92.5	90.0	90.5
BC20-211	BLQ	95.7	100.0	93.7	94.5	88.0	100.8
Percentage of lots met the criteria		100.0%		100.0%		100.0%	

RA	Blank	LQC	HQC	LQC	HQC	LQC	HQC
BRH502704	BLQ	87.7	108.3	94.3	97.3	97.3	87.3
BRH502724	BLQ	97.0	97.0	109.3	101.3	93.7	84.8
BRH502706	BLQ	NC	118.3	114.3	93.5	105.0	94.5
BRH502707	BLQ	98.7	98.0	118.7	97.8	112.3	99.8
BRH502708	BLQ	101.7	111.3	104.3	96.5	98.0	96.3
BRH502709	BLQ	132.0	110.5	134.3	124.0	127.3	107.0
BRH502710	BLQ	109.0	114.8	97.0	83.8	97.0	82.5
BRH502711	BLQ	100.7	98.8	99.0	95.8	105.3	100.3
BRH502712	BLQ	100.0	107.5	107.3	108.0	98.0	93.5
BRH502713	BLQ	115.7	105.8	110.7	104.3	98.0	87.3
BRH502714	BLQ	90.3	95.0	95.3	86.5	86.7	90.0
BRH502715	BLQ	100.3	95.5	116.0	93.8	105.0	93.5
BRH502716	BLQ	130.0	129.3	110.3	81.8	98.0	96.8
BRH502717	BLQ	104.0	88.3	100.3	85.8	97.3	85.8
BRH502718	BLQ	103.0	102.0	103.0	94.3	105.0	100.8
BRH502719	BLQ	88.7	91.3	108.0	85.8	87.7	99.0
BRH502720	BLQ	114.7	118.8	101.3	76.3	93.3	82.0
BRH502721	BLQ	94.0	96.8	96.0	92.0	80.7	84.8
BRH502722	BLQ	103.0	100.3	115.7	97.8	99.7	81.5
BRH502723	BLQ	106.3	100.0	111.0	95.5	96.3	93.3
Percentage of lots met the criteria		85.0%		95.0%		95.0%	

Additionally, matrix selectivity was examined in two individual lots each from low, mid, and high levels of hemolyzed and hyper-lipemic serum samples. The results demonstrated no interference from hemolysis (Table 3A) but hyperlipidemia (Table 2B).

Table 2B. Matrix Selectivity (% Recovery) of Rituximab in Human Serum

			Rituximab-EU		Rituximab-Pfizer	
Hemolyzed Lot (Hemoglobin level shown)		Blank	LQC	HQC	LQC	HQC
140 mg/dL	BRH648220	BLQ	94.7	107.8	93.7	106.5
140 mg/dL	BRH648221	BLQ	97.7	105.8	98.0	106.0
275 mg/dL	BRH648222	BLQ	100.0	108.0	100.7	107.8
275 mg/dL	BRH648223	BLQ	104.0	111.3	104.0	102.8
1100 mg/dL	BRH648224	BLQ	89.0	115.5	89.0	106.3
1100 mg/dL	BRH648225	BLQ	99.7	107.3	99.3	107.3
Percentage of lots met the criteria:				100.0%		100.0%

Hyper-lipemic Lot		Blank	LQC	HQC	LQC	HQC
Low level	BRH648220	BLQ	117.7	132.3	109.7	110.8
Low level	BRH648221	BLQ	92.7	113.5	93.0	104.3
Medium level	BRH648222	BLQ	486.7	117.8	220.7	116.8
Medium level	BRH648223	BLQ	110.0	90.7	104.3	90.7
High level	BRH648224	BLQ	NR	121.3	NR	110.3
High level	BRH648225	BLQ	105.3	119.3	105.7	109.3
Percentage of lots met the criteria:				50.0%		66.7%

Anti-drug antibody (ADA) can potentially interfere with the PK assay. The potential ADA interference were assessed by spiking the appropriate Positive Control (affinity purified polyclonal rabbit anti-Rituximab antibody) levels into the targeted Rituximab-EU, Rituximab-US and Rituximab-Pfizer LQC and HQC concentrations, compared to non ADA-spiked LQC and HQC concentrations. Because surrogate Positive Control (affinity purified polyclonal rabbit anti-Rituximab antibody) used in this assessment would not reflect clinical incurred ADA, therefore no criteria was set-up for this assessment. The assay was found not to be interfered with the presence of up to 500 ng/mL of ADA Positive Controls (Table 3).

Table 3. Interference of ADA in Rituximab Assay (% Recovery)

	Rituximab-EU		Rituximab-US		Rituximab-Pfizer	
PC	LQC	HQC	LQC	HQC	LQC	HQC
0 ng/mL	97.3	92.2	98.0	102.5	109.0	89.7
500 ng/mL	85.7	90.0	93.7	102.0	109.0	106.5
1,000 ng/mL	78.0	86.7	88.3	108.0	92.3	99.2
2,500 ng/mL	63.7	97.2	71.0	103.8	77.3	99.2
5,000 ng/mL	34.3	96.0	35.3	103.3	44.7	98.0
10,000 ng/mL	NA (BLQ)	86.5	NA (BLQ)	97.7	NA (BLQ)	90.2

Recoveries within 80-120% are considered acceptable without interference.

The dilution linearity was established in serum for Rituximab-EU, Rituximab-US and Rituximab–Pfizer. Stabilities of Rituximab-EU and Rituximab Pfizer were assessed. It was found that the analytes were stable in serum for at 18 hours at room temperature, at least 5 freeze/thaw cycles and up to 12 months long-term frozen storage (Data not shown).

CONCLUSIONS

- An ELISA assay was developed and validated for the analysis of both rituximab innovator and rituximab biosimilar. The assay exhibited acceptable precision, accuracy, selelctivity and specificity within the quantiative range, therefore was found acceptable to quantify rituximab in human serum to support clinical trials.

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