GYROS PROTEIN Technologies

Gyrolab® Assays

Remsima™(infliximab) PK Assay

INTRODUCTION

Remsima (infliximab) is an anti-inflammatory biopharmaceutical that belongs to the group of TNF-α (tumor necrosis factor-alpha) inhibitors. Remsima is a biosimilar to the original monoclonal antibody infliximab (Remicade®).

To support clinical PK studies we have developed a three-step sandwich Gyrolab PK Assay to determine Remsima in human serum samples. An MRD of 1:500 gives a broad analytical range with and approximate LLOQ of 200 ng/mL and ULOQ of 200 µg/mL in neat serum/plasma. Use of this protocol on Gyrolab systems will reduce time to market and increase productivity while maintaining quality requirements.

ASSAY DESIGN

The assay was set up as a three-step sandwich assay with biotinylated human TNFα as a capture molecule and a mouse anti-human IgG Fc fragment as a detection molecule. The same assay design can be used for Remicade since Remsima is a biosimilar to Remicade.

Mouse anti-human IgG Fc

Remsima

hTNFα

ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust three-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 200 ng/mL unit to 200 µg/mL (Table 1). The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in duplicate in three runs, was <20% (Table 2).

Table 1 Estimated Assay Range in neat serum, based on three runs

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 200	~ 200 000

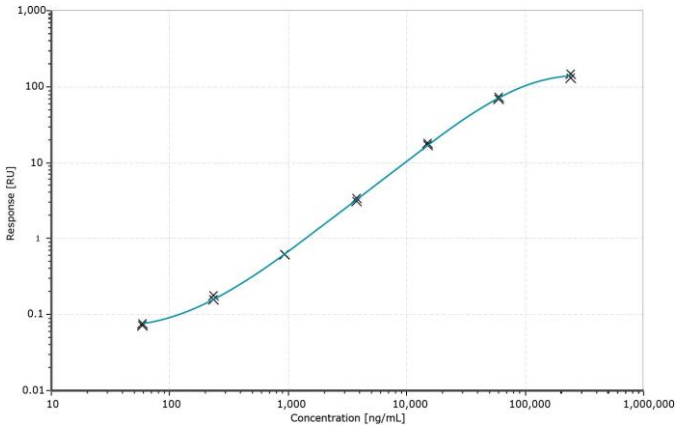

Figure 1 Standard curve in 0.5% serum. Concentrations in neat serum

Table 2 Accuracy and precision data of QC samples in neat serum, n= number of runs

QC	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	Inter Run CV% (n=3)	Average Intra Run CV% (n=3)	Average Total Error% (n=3)
LLOQ	200	229	10	6.8	21
LQC	500	445	7.6	7.8	19
MQC	80000	87917	6.2	2.5	12
HQC	180000	185833	6.0	6.0	9.2
ULOQ	200000	219500	2.7	1.2	11

Selectivity

Selectivity was established by spiking 200 ng/mL of the drug in human serum samples.

Table 3 Selectivity spiked samples

Sample	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	CV%	Average Bias%
1	200	189	13	-5.3
2	200	211	4.6	5.7
3	200	191	10	-4.3
4	200	218	10	9.1
5	200	190	21	-5.0
6	200	223	16	12
7	200	249	12	24
8	200	198	1.2	-0.76
9	200	245	7.4	23

MATERIALS AND METHODS

The assay was developed on Gyrolab xP using Gyrolab Bioaffy 1000 CD. The assay was set up using a 3-step format with two wash solutions (1000-3W-006-A). The assay buffer was Rexxip H with an MRD of 1:500. hTNFα (CYT-223) from Prospec was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 210 nM and 490 nM biotinylated BSA (B-2007) from Vector Laboratories diluted in PBST.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the mouse anti-human IgG Fc JCD-10 from Southern Biotech, diluted to 6.25 nM in Rexxip F. The assay standard used was Remsima from Celltrion Healthcare. The standard was prepared in human serum diluted in Rexxip H.

Recommendations

When developing this assay for a specific drug development purpose, it is important to screen matrices and assess backgrounds, in particular for the specific disease matrices. Parameters, such as LLOQ need to be validated in-house. Data given in this document should only be considered as a guidance.

For additional support contact your local Field Application Support