GYROS PROTEIN Technologies

Gyrolab® Assays

Actemra® (tocilizumab) PK Assay

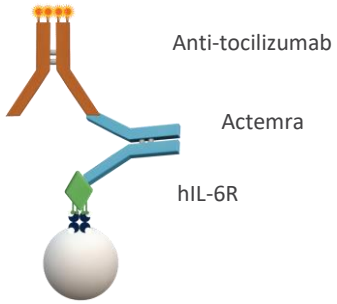
INTRODUCTION

Actemra (tocilizumab) is an anti-inflammatory biopharmaceutical used in the treatment of rheumatoid arthritis and similar inflammatory conditions. Actemra is a humanized antibody belonging to the group of IL-6R (interleukin 6 receptor) inhibitors.

We have developed a three-step bridging Gyrolab PK assay to determine Actemra levels in human serum samples. An MRD of 20 gives a broad analytical range with an LLOQ on the Gyrolab® Bioaffy™ 1000 CD of 60 ng/mL, and an LLOQ on the Gyrolab® Bioaffy™ 4000 CD of 20 ng/mL in neat serum. On both CD types the ULOQ was set at 20 000 ng/mL in neat serum. 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 bridging assay with biotinylated human IL-6R as a capture molecule and a recombinant human anti-tocilizumab labeled with Alexa Fluor® 647 as a detection molecule.



ASSAY PERFORMANCE

Dynamic range, accuracy and precision

Robust 3-log standard curves (Figure 1) were generated over three runs each using two different Gyrolab Bioaffy CDs, achieving the assay ranges shown in Table 1. The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in triplicate in three runs, was <20% (Table 2) for both CD types.

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

CD Type	LLOQ (ng/mL)	ULOQ (ng/mL)
Gyrolab Bioaffy 1000	~ 60	~ 20 000
Gyrolab Bioaffy 4000	~ 20	~ 20 000

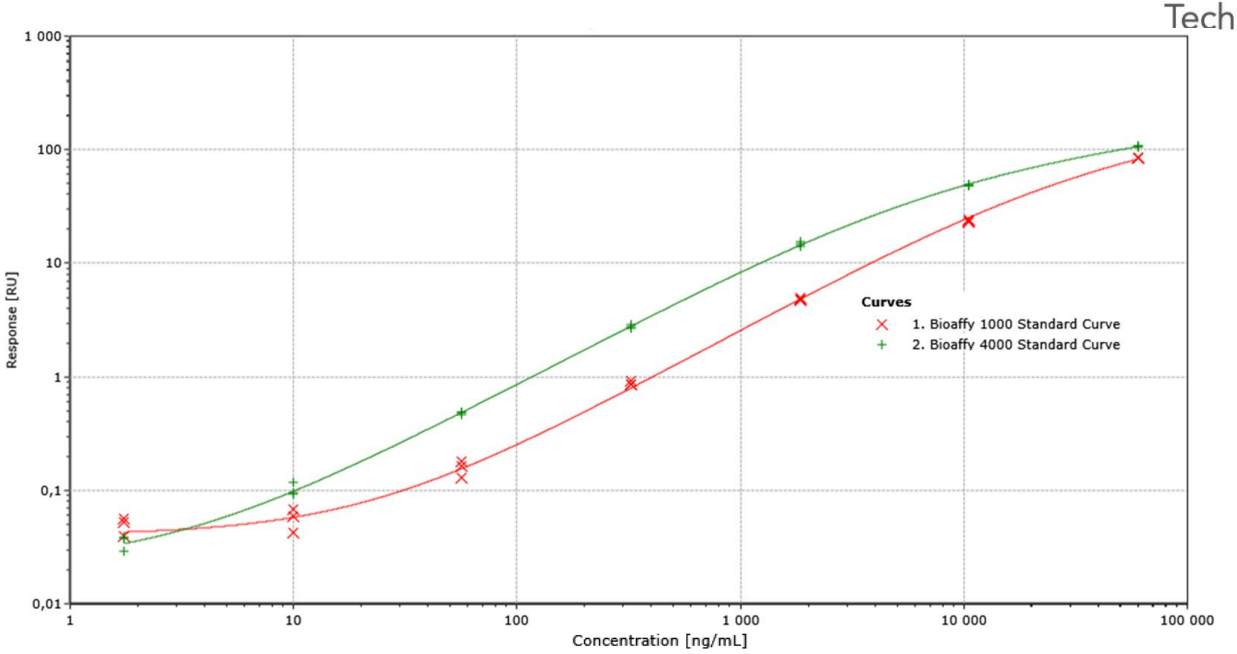


Figure 1 Standard curves in Rexxip H with 5% serum. Concentrations in neat serum

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

CD Type	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)
Gyrolab Bioaffy 1000	60	62	20	20	37
	180	180	7.7	9.2	16
	650	670	5.4	7.1	12
	8 000	8 200	1.9	4.2	6.7
	20 000	20 000	3.9	3.2	7.0
Gyrolab Bioaffy 4000	20	19	12	16	24
	60	58	4.9	5.7	9.2
	650	700	4.3	5.1	13
	8 000	8 300	3.5	3.8	7.9
	20 000	19 000	4.7	4.2	11

Selectivity

Selectivity was established by spiking the drug at the LLOQ level in human serum samples. All samples measured <LLOQ when analyzed unspiked.

Table 3 Selectivity spiked samples

CD Type	Sample	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	CV (%)	Average Bias (%)
Bioaffy 1000	1	60	67	21	12
	2	60	62	23	3.8
	3	60	61	6.4	0.9
	4	60	63	14	5.5
	5	60	59	11	-1.3
	6	60	48	26	-19
	7	60	68	7.8	13
	8	60	69	17	16
	9	60	59	21	-1.8
	10	60	73	24	22
Bioaffy 4000	1	20	20	8.2	-1.6
	2	20	23	24	16
	3	20	18	5.6	-10
	4	20	20	8.2	0.1
	5	20	21	16	5.5
	6	20	20	8.7	-1.0
	7	20	18	4.2	-8.4
	8	20	24	14	19
	9	20	20	8.0	0.5
	10	20	19	17	-2.5

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab Bioaffy 1000 CD and Gyrolab Bioaffy 4000 CD. The assay was set up using 3-step Gyrolab methods with two wash solutions (1000-3W-006-A and 4000-3W-001-A) and a 1% PMT setting. The assay buffer was Rexxip H with 5% human serum. Human IL-6R (Abcam, ab167742) was biotinylated as detailed below and used in a concentration of 175 nM, diluted with PBS-T containing 525 nM Biotin-BSA.

Recombinant IL-6R protein and BSA (Sigma, A3803) as supplied was reconstituted to 1 mg/mL with deionized water. The reconstituted protein was then reacted with 12 molar equivalents of Biotin-XX, SE (Thermo Fisher Scientific, B1606) as a 4 mg/mL solution freshly prepared using DMSO, and the resulting mixture vortexed briefly, before being roller-mixed for 1 hour at 20°C, in the dark. The conjugate was purified by desalting into 50 mM phosphate 150 mM NaCl pH 6.7 buffer via zeba 0.5 mL spin desalting column, and UV analyzed to determine product concentration.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was recombinant human anti-toxcilizumab HCA253 from BioRad, diluted to 35 nM in Rexxip F. The assay standard used was the humanized IgG1 monoclonal antibody tocilizumab (Actemra) from Roche. The standard was prepared in 5% human serum diluted in Rexxip H.

Summary table

Capture	175 nM biotinylated IL-6R (ab167742, Abcam) + 525 nM biotinylated BSA (A3803, Sigma-Aldrich) in PBS-T	
Detection	Alexa Fluor 647 labeled anti-toxcilizumab (HCA253, Bio-Rad), 35 nM in Rexxip F	
Analyte	Actemra (Roche) in Rexxip H with 5% human serum	
CD-type	Gyrolab® Bioaffy™ 1000 CD	Gyrolab® Bioaffy™ 4000 CD
Method	1000-3W-006-A	4000-3W-001-A
Full CD run time	Approx. 70 minutes	Approx. 90 minutes
Sample volume required (triplicate)	10 µL	16 µL
Expected dynamic range	Approximately 60 – 20 000 ng/mL in neat serum	Approximately 20 – 20 000 ng/mL in neat serum
Minimum required dilution	1-in-20	
Wash buffer for needles	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH 11	
PMT-setting	1%	

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 should be validated in-house. Data given in this document should only be considered as guidance.

For additional support contact your local Field Application Support