GYROS PROTEIN Technologies

Gyrolab® Assays

Actemra® (tocilizumab) ADA Assay

INTRODUCTION

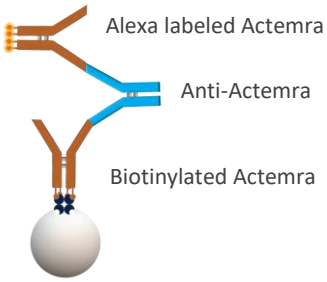
Actemra (tocilizumab) is an anti-inflammatory biopharmaceutical used in the treatment of rheumatoid arthritis and similar inflammatory conditions. Actemra is a humanized antibody belonging to the group of IL6R (interleukin 6 receptor) inhibitors.

Anti-drug antibodies (ADA) can be generated by patients when biopharmaceuticals are administered. The presence of ADA can inhibit biopharmaceuticals binding the target, impact the PK profile, and affect safety and efficacy. Detection and characterization of ADA is an integral part of biopharmaceutical drug development.

We have developed a homogenous bridging Gyrolab ADA assay to determine levels of anti-Actemra antibodies in human serum samples. An MRD of 10 gives a specific and drug tolerant assay. 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 homogenous bridging assay following acid dissociation, 0.5 M Glycin-HCl pH 2.6, with biotinylated Actemra as a capture molecule and Actemra labeled with Alexa Fluor® 647 as a detection molecule. The positive control used for this assay was a human anti-idiotypic mAb from a commercial source.



ASSAY PERFORMANCE

Assay Sensitivity

The assay sensitivity was estimated from the screening cut point as <0.125 ng/mL of positive control antibody, see Figure 1. Due to the qualitative nature of ADA assays the assay sensitivity is not an absolute concentration of anti-drug antibody, and may be different in a clinical setting. Use of a surrogate positive control is, however, best industry practice and accepted by regulatory agencies.

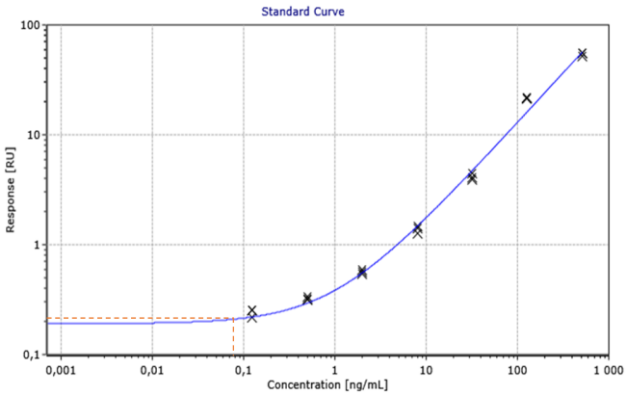


Figure 1 Positive control titration in Rexxip ADA with 10% serum. Concentrations in neat serum.

Assay screening cut point

A floating cut point with 5% false-positive rate was estimated from 18 healthy mixed gender individuals, analyzed over three runs. This would not constitute a validated cut point assessent but was estimated from the principles outlined in Shankar et al. (Recommendations for the Validation of Immunoassays Used for Detection of Host Antibodies Against Biotechnology Products. Journal of Pharmaceutical and Biomedical Analysis 2008;48;1267-1281). The responses for individuals is shown in Figure 2. The normalization factor was set at 1.1 from the responses of this population.

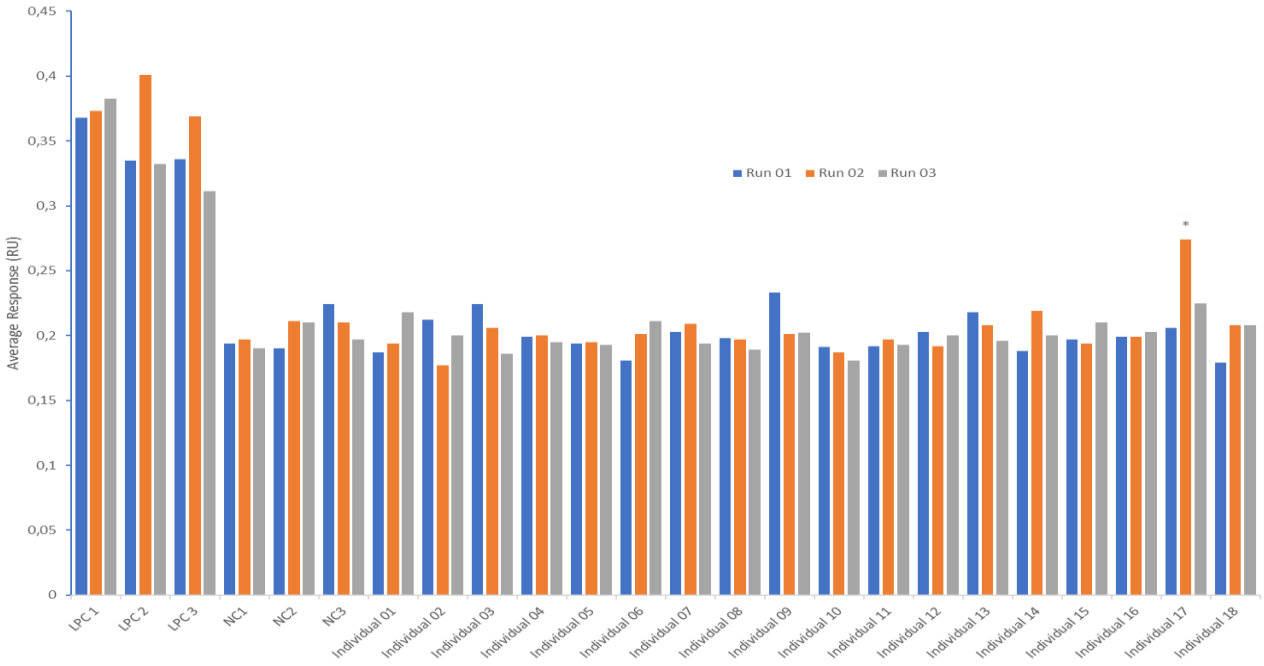


Figure 2 Average responses from Low Positive Controls (LPC1 – LPC3) at 1 ng/mL, Negative Controls (NC1 – NC3) and individual human serum samples (Individual 01 – 18) in Rexxip ADA containing 10% human serum. Concentrations in neat serum. Outliers identified by Grubbs Test marked with *.

Screening assay precision

The assay precision for the positive control across three runs at the Low Positive Control (LPC 1.0 ng/mL) and High Positive Control (HPC 100 ng/mL) was ≤19% and precision for the pooled negative control over the same three runs was 5.8%.

Table 1 Precision assessment of the screening assay over three runs

Parameter	Negative Control (Pooled human serum)	Low Positive Control (1.0 ng/mL)	High Positive Control (100 ng/mL)
Average response	0.203 RU	0.356 RU	13.4 RU
Intra-assay precision	≤23%	≤11%	≤4.4%
Inter-assay precision	5.8%	8.1%	19%

Confirmatory assay

Mixed gender individuals were analyzed in three runs using mastermix with 8 mg/mL Actemra and without Actemra to determine the level of inhibition equivalent to a 1% false positive rate. The confirmatory cut point was determined from this set of individuals as 32.6% inihibition in the presence of mastermix with 8 mg/mL Actemra.

Drug tolerance

Drug tolerance was assessed by titrating concentrations of Actemra in the presence of 100 ng/mL ADA positive control in pooled human serum. At 100 ng/mL of positive control drug tolerance was estimated at 870 µg/mL of Actemra as this is where the curve intersects the screening cut point, see

Figure 3. Full inhibition of 100 ng/mL of ADA positive control was achieved at >1 280 µg/mL of Actemra, equivalent to average response of assay blank at 0.221 RU; inhibition results are shown in Table 2. The expected trough level of Actemra in human serum is 50 µg/mL.

Table 2 Drug tolerance assessment in the presence of 100 ng/mL positive control

Actemra Conc. (µg/mL)	100 ng/mL Positive Control	
	Average response (RU)	% Inhibition
0	11.3	N/A
10	7.16	36.6
20	4.88	56.8
40	3.42	69.7
80	1.77	84.3
160	0.904	92.0
320	0.442	96.1
640	0.274	97.6
1 280	0.224	98.0

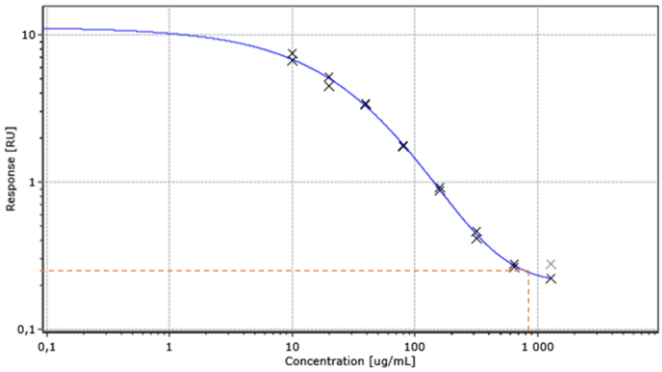


Figure 3 Titration of Actemra in Rexxip ADA with 10% serum and 100 ng/mL of positive control. Concentrations in neat serum.

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab Mixing CD 96. The assay was set up using a 1-step Gyrolab method with two wash solutions (Mixing96-1W-003-A) and a 5% PMT setting. The assay buffer was Rexxip ADA with 10% human serum. The acid dissociation step was performed using 0.5 M Glycin-HCl pH 2.6. A bridging assay format was used with equal concentrations of capture and detection reagents in 1 M Tris-HCl pH 8.

The capture reagent was the humanized IgG1 monoclonal antibody tocilizumab (Actemra) from Roche. 200 µg of Actemra (2 mg/mL in PBS) was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide), with a 1:10 challenge ratio of biotin. Capture reagent was used in mastermix at a concentration of 5 µg/mL in Rexxip ADA.

The detection reagent was the humanized IgG1 monoclonal antibody tocilizumab (Actemra) from Roche. 200 µg of Actemra (2 mg/ml in PBS) was labeled with one vial of Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide). The detection reagent was used in mastermix at a concentration of 5 µg/mL in Rexxip ADA.

The positive control used was a human anti-tocilizumab monoclonal antibody, clone AbD21346_hIgG1, sourced from Bio-Rad (HCA255), diluted in Rexxip ADA with 10% human serum.

Summary table

Capture	Biotin labeled tocilizumab (Actemra, Roche), 10:1 molar challenge ratio
Detection	Alexa Fluor 647 labeled tocilizumab (Actemra, Roche)
Mastermix	5 µg/mL detection reagent and 5 µg/mL capture reagent in 1 M Tris-HCL pH 8
Acidic Buffer	0.5 M Glycin-HCl pH 2.6
Analyte	Human anti-tocilizumab monoclonal antibody (HCA255; Bio-Rad) in Rexxip ADA with 10% human serum
CD-type	Gyrolab Mixing CD 96
Method	Mixing96-1W-003-A
Wash buffer for needles	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH 11
PMT-setting	5%
Negative Control inter assay precision	5.8%
Suggested Positive Control concentration	LPC: 1.0 ng/mL HPC: 100 ng/mL
Confirmatory drug concentration	8 mg/mL
Assay sensitivity	<0.125 ng/mL*
Drug tolerance	870 µg/mL*
Screening Cut point	1.1 x Mean NC RU*
Confirmatory Cut point	32.6%*

* These values are dependent on the pool and individuals used in the assay validation.

Recommendations

When developing this assay for a specific drug development purpose, it is important to perform in-house screening of matrices and determine population screening cutpoint correction factor. Parameters, such as sensitivity and positive control concentrations should be validated in-house. Data given in this document should only be considered as guidance. Additional details on recommendations for optimizing an ADA method on the Gyrolab system can be found via the Gyrolab User Zone.

For additional support contact your local Field Application Support