GYROS PROTEIN Technologies

Gyrolab® Assays

Remsima® (infliximab) ADA Assay

INTRODUCTION

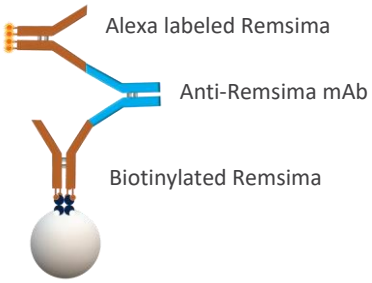
Remsima® (infliximab) is an anti-TNF alpha monoclonal antibody that is administered to patients with autoimmune disorders such as rhemathoid arthitis, psoriasis, Chrons disease and ulcerative colitis.

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

We have developed a homogenous bridging Gyrolab ADA assay with automated acid dissociation to determine levels of anti-Remsima antibodies in human serum samples. An MRD of 10 in Rexxip® ADA 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 with acid dissociation using 0.5 M Glycin-HCl pH 2.6, and biotinylated Remsima as a capture molecule and Remsima labeled with Alexa Fluor® 647 as a detection molecule. The positive control used for this assay was a human anti-idiotypic mAb for Infliximab from BioRad.



ASSAY PERFORMANCE

Assay Sensitivity

The assay sensitivity was estimated from the screening cut point as 6.25 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, industry practice and accepted by regulatory agencies.

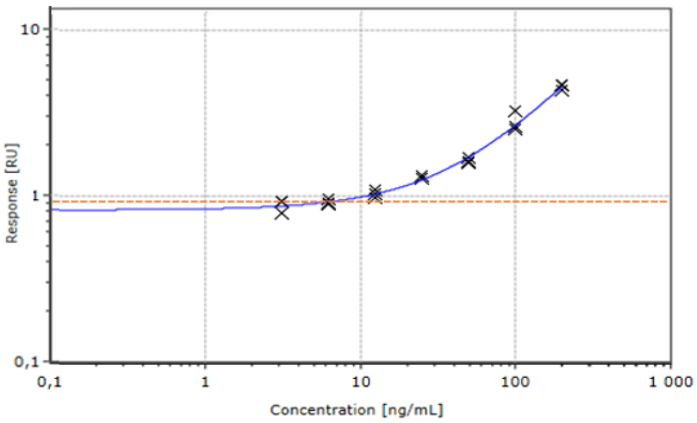


Figure 1. Positive control titration in Rexxip ADA resulting in 10% serum. Concentrations in neat serum. Screening cutpoint was 0.910 RU.

Assay screening cut point

A floating cut point with 5% false-positive rate was estimated from 51 treatment naïve healthy mixed gender individuals, analyzed once across three independent runs. The normalized responses (Mean Individual RU/Assay Mean NC RU) of the individuals were found to be normally distributed (Shapiro-Wilkes a=0.05) and was therefore not log transformed. No values were found to be outliers by Grubbs test. This would not constitute a validated cut point assessment 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 average responses for individuals and positive controls are shown in figure 2. The floating cut point was set as Mean NC + 0.062.

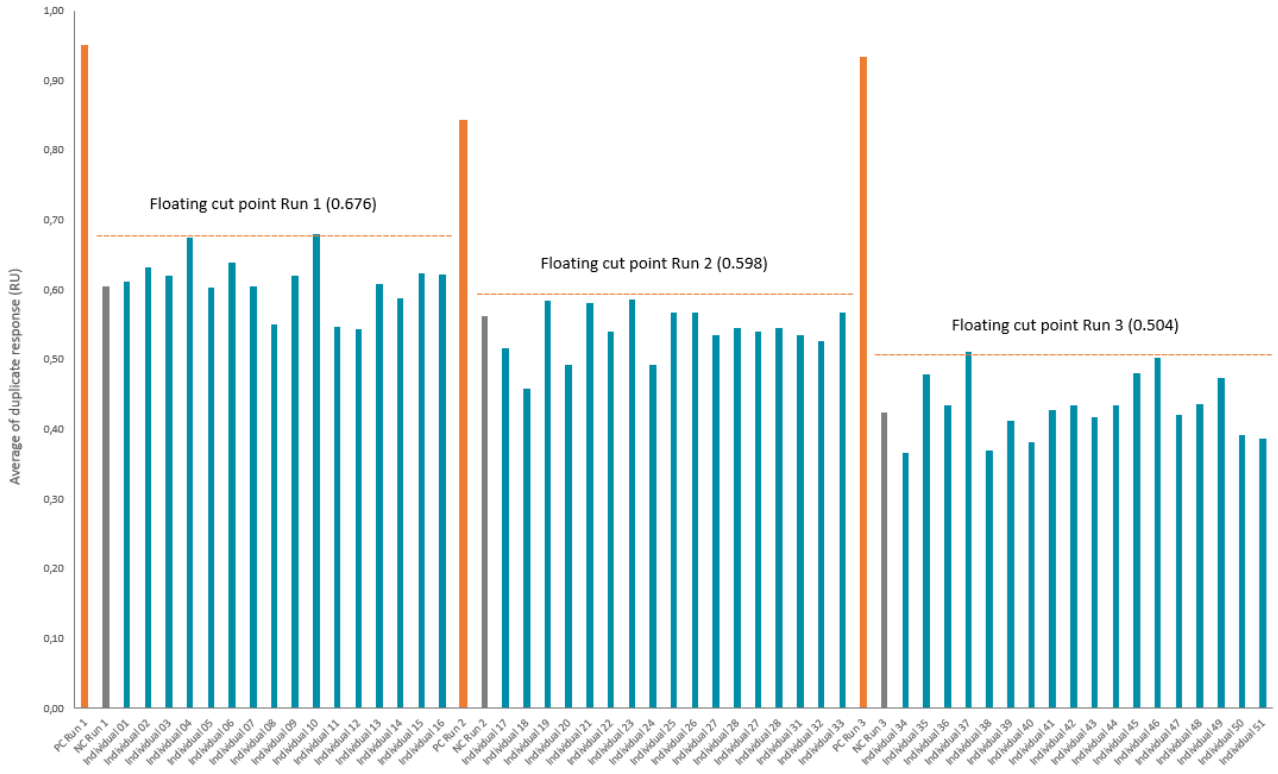


Figure 2. Average response values in duplicates of Negative Control (NC), Positive Controls (PC) at 20 ng/mL in neat serum and individual human serum samples (Individual 01 – 51) in Rexxip ADA, for a final concentration of 10% human serum. The Individuals 10 and 37 were screened as positive.

Screening assay precision

The assay precision across three runs for the positive control at 20 ng/mL was 13.6% and precision for the pool negative control was 17.3%, see Table 1.

Table 1. Precision assessment of the screening assay over three runs

Parameter	Negative Control (Pooled human serum)	Positive Control (20 ng/mL)
Average response	0.536 RU	0.894 RU
Intra-assay precision	≤6.2%	≤9.4%
Inter-assay precision	17.3%	13.6%

Confirmatory assay

Mixed gender individuals were analyzed in three runs following a 1-in-10 MRD using assay diluent with 200 µg/mL Remsima and without Remsima 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 23.2% inihibition in the presence of Rexxip ADA with 200 µg/mL Remsima. None of the individuals that screened positive were confirmed positive.

Drug tolerance

Drug tolerance was assessed by titrating concentrations of Remsima in the presence of 100 ng/mL ADA positive control in pooled human serum. At 100 ng/mL of positive control drug tolerance was set at 80 µg/mL of Remsima as this is the concentration above which the positive control response falls below the screening cut point, see Figure 3. A plateau in inhibition of 100 ng/mL of ADA positive control was achieved at 320 µg/mL of Remsima, equivalent to average response of assay blank at 0.850 RU; inhibition results are shown in Table 2. The anticipated trough level of Remsima in human serum is ≤ 5 µg/mL.

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

Remsima Conc. (µg/mL)	100 ng/mL Positive Control	
	Average response (RU)	% Inhibition (Blank subtracted)
0	2.498	N/A
10	2.070	26
20	1.756	46
40	1.387	68
80	1.069	88
160	0.936	96
320	0.850	101
640	0.803	104
1 280	0.808	104

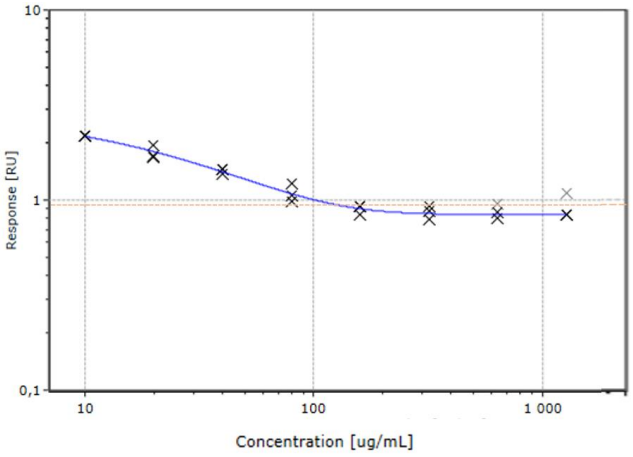


Figure 3. Titration of Remsima in Rexxip ADA with 10% serum and 100 ng/mL of positive control. Concentrations in neat serum. The floating cut point for this run was 0.910 RU.

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 25% PMT setting. The assay buffer was Rexxip® ADA with 10% human serum, the confirmatory assay buffer was Rexxip ADA with 10% human serum and 200 µg/mL of Remsima. The acid dissociation step was performed using 0.5 M Glycin-HC,l 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 IgG monoclonal antibody Infliximab (Remsima) from Celltrion Healthcare. 100 µg of Remsima (1 mg/mL in PBS) was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide), with a 12:1 challenge ratio of biotin. Capture reagent was used in mastermix at a concentration of 2 µg/mL in Rexxip ADA.

The detection reagent was the humanized IgG monoclonal antibody Infliximab (Remsima) Celltrion Healthcare. 100 µg of Remsima (1 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 2 µg/mL in Rexxip ADA.

The positive control used was a human anti-infliximab monoclonal antibody, clone AbD17841_hIgG1, sourced from Bio-Rad (HCA213), diluted in Rexxip ADA with 10% human serum.

Summary table

Capture	Biotin labeled infliximab (Remsima, Celltrion Healthcare), 12:1 molar challenge ratio
Detection	Alexa Fluor 647 labeled infliximab (Remsima, Celltrion Healthcare)
Mastermix	2 µg/mL capture reagent and 2 µg/mL detection reagent in 1 M Tris-HCL, pH 8
Acidic Buffer	0.5 M Glycin-HCl, pH 2.6
Analyte	Human anti-infliximab monoclonal antibody (HCA213; 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	25%
Negative Control inter assay precision	17.3%
Suggested Positive Control concentration	PC: 20 ng/mL
Confirmatory drug concentration in Rexxip ADA with 10% human serum	200 µg/mL
Assay sensitivity	6.25 ng/mL*
Drug tolerance	80 µg/mL*
Screening Cut point	0.062 + Mean NC RU*
Confirmatory Cut point	23.2% inhibition*

* 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