Gyrolab® Assays

Enbrel® (Etanercept) ADA Assay

INTRODUCTION

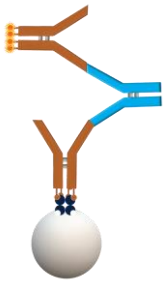
Enbrel (Etanercept) is a biopharmaceutical that treats autoimmune diseases such as rheumatoid arthritis and psoriasis by interfering with immune regulator tumor necrosis factor alpha (TNF-α). Enbrel is a recombinant fusion protein which fuses an IgG1 antibody with the TNF-α receptor causing inhibition of the TNF receptor.

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 ADA response is an integral part of biopharmaceutical drug development.

We have developed a homogenous bridging Gyrolab ADA assay with automated acid dissociation to determine the levels of anti-Enbrel in human serum samples. A Minimal Required Dilution (MRD) of 10 in Rexxip® ADA Buffer gives a specific and drug tolerant assay. Use of this protocol on Gyrolab systems will reduce time to market and increase productivity whilst maintaining quality requirements.

ASSAY DESIGN

The assay was set up as a homogenous bridging assay following acid dissociation by 0.5 M Glycine-HCl, pH 2.6 with biotinylated Enbrel as a capture molecule and Enbrel labeled with Alexa Fluor® 647 as a detection molecule. The positive control used was a human recombinant monoclonal anti-etanercept antibody, clone AbD25940 from Bio-Rad.



Alexa labeled Enbrel

Anti-Enbrel

Biotinylated-Enbrel

ASSAY PERFORMANCE

Sensitivity

The assay sensitivity was estimated from the screening cut point as 9.38 ng/mL of positive control antibody, see

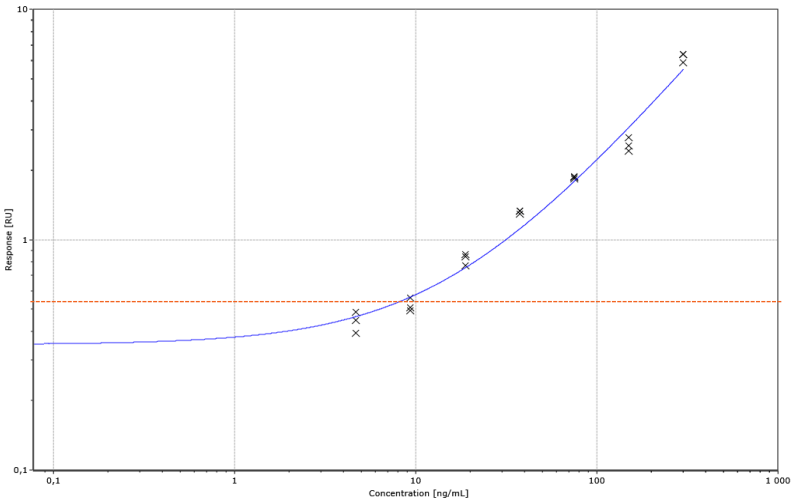


Figure 1. Positive control dilution in Rexxip ADA buffer with 10% serum. Screening cut point = 0.447 RU. Concentrations in neat serum.

Assay Screening Cut Point

A floating cut point with 5% false-positive rate was estimated from a population of 54 healthy mixed gender individuals. The normalized responses (Mean Individual RU / Assay Mean NC RU) of the individuals were found to be normally distributed (Shapiro-Wilkes, α=0.05). Outliers were determined by Rosner's Extreme Studentized Deviate test (p=0.05) for multiple outliers (two-sided). No values were found to be outliers.. Due to the qualitative nature of ADA assays the sensitivity

is not an absolute concentration of anti-Enbrel antibody, and may be different in a clinical setting. Use of surrogate positive control is, however, best industry practice and accepted by regulatory agencies.

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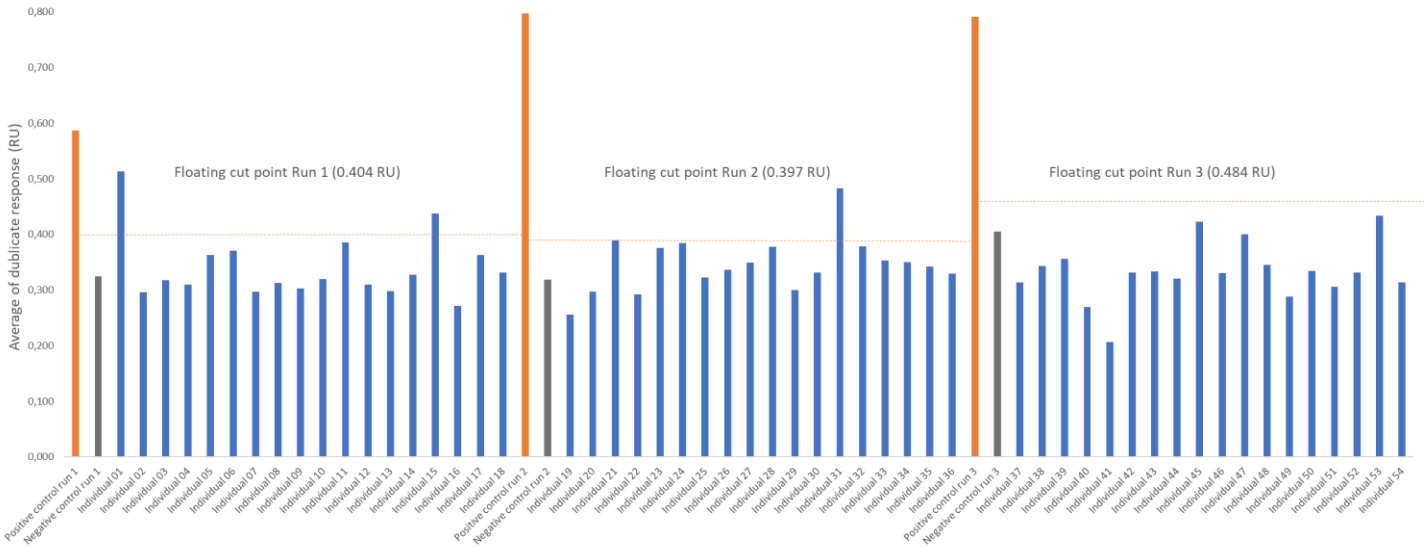
The average responses for individuals and positive controls are shown in Figure 2. The floating cut point was



set as Mean NC RU + 0.079 RU.

These screens 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).

Figure 2. Average of duplicate responses (RU) from Negative Control (NC) Positive Controls (PC) at 50 ng/mL and individual human serum samples (Individual 01 – 54) in Rexxip ADA buffer, for a final concentration of 10% human serum. Concentrations of PC in neat serum.

Screening assay precision

Table 1 presents the assay precision across three runs for the positive and negative control samples. The precision for the positive control assay at 50 ng/mL was 15.6% CV. The precision for the pooled negative control over the same three runs was 15.3% CV, as shown in table 1.

Table 2. Screening assay precision across three runs

Parameter	Negative Control (Pooled Human Serum)	Positive Control (Pooled Human Serum)
Average response	0.349 RU	0.725 RU
Intra-assay precision	≤11%	≤20%
Inter-assay precision	15.3%	15.6%

Confirmatory assay

54 mixed gender individuals were analyzed over three runs following a 1-in-10 MRD using assay diluent with 20 µg/mL Enbrel and without Enbrel 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 46.7% inhibition in the presence of Rexxip ADA with 50 µg/mL Enbrel. One of the individuals that screened positive was confirmed positive.

Drug tolerance

Drug tolerance was assessed by serially diluting Enbrel 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 to 160 ng/mL Enbrel. This is the first concentration above which the positive control response falls below the screening cut point. For the acid dissociation assay, a plateau in inhibition of 100 ng/mL of ADA positive control was achieved at around 320 ng/mL Enbrel, equivalent to average response of assay blank at 0.296 RU.

The anticipated trough level of Enbrel in human serum is ≤ 3 µg/mL.

Table 1 Drug tolerance assessment in the presence of 100 ng/mL positive control

Enbrel Conc. (µg/mL)	100 ng/mL Positive Control	
	Average response (RU)	% Inhibition
0	14.5	N/A
2.5	5.25	68.0
5	3.17	83.3
10	3.61	80.1
20	2.09	91.3
40	1.11	98.5
80	0.56	102.5
160	0.34	104.2
320	0.31	104.3

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab Mixing CD 96 using a 1-step assay 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 buffer was Rexxip ADA with 10% human serum and 50 µg/mL of Enbrel. A bridging assay format was used with equal concentrations of capture and detection reagents in 1 M Tris-HCl, pH 8.

The acid dissociation step was performed using 0.5 M Glycin-HCl, pH 2.6.

Enbrel from Pfizer was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide), with a 12:1 challenge ratio of biotin. Capture reagent was used in Master Mix at a concentration of 1 µg/mL in Rexxip ADA.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was 100 µg Enbrel (1 mg/mL in PBS) from Pfizer.

The positive control a monoclonal human anti-etanercept (Enbrel) IgG4, clone AbD25940, sourced from Bio-Rad, diluted in Rexxip ADA with 10% human serum.

Summary table

Capture	Biotin labeled Enbrel (Pfizer) 12:1 molar challenge ratio
Detection	Alexa Fluor 647 labeled Enbrel (Pfizer)
Master Mix	1 µg/mL detection reagent and 1 µg/mL capture reagent in Rexxip ADA
Acidic Buffer	0.5 M Glycine-HCl, pH 2.6*
Analyte	Monoclonal human anti-etanercept (Enbrel) antibody (cat. no.: HCA277, BioRad) 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	15.3%
Suggested Positive Control concentration	50 ng/mL**
Confirmatory drug concentration in Rexxip ADA with 10% human serum	20 µg/mL
Assay sensitivity	9.38 ng/mL
Drug tolerance	160 µg/mL***
Screening Cut point	0.079 + Mean NC RU***
Confirmatory Cut point	46.7% inhibition***

* This assay can also be set up as an overnight assay without acid dissociation. Please contact your local Field Application Support for additional information.

** Recommended Positive Control concentration updated following sensitivity assessment.

*** 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 cut point 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

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