GYROS PROTEIN Technologies

Gyrolab® Assays

Herceptin® (Trastuzumab) ADA Assay

INTRODUCTION

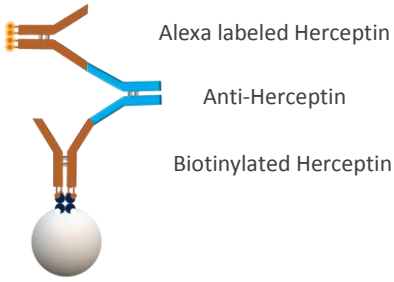
Herceptin (Trastuzumab) is an immunotherapy biopharmaceutical that is used to treat breast cancer and stomach cancer. Herceptin is a humanized antibody of IgG1 isotype that targets human epidermal growth factor receptor 2 (HER2), inducing an immune-mediated response that causes internalization and downregulation of HER2.

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-Herceptin in human serum samples. A Minimal Required Dilution (MRD) of 1-in-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 whilst maintaining quality requirements.

ASSAY DESIGN

The assay was set up as a homogenous bridging assay following acid dissociation, 0.5 M Glycine-HCl pH 2.6, with biotinylated Herceptin as a capture molecule and Herceptin 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

Sensitivity

The assay sensitivity was estimated from the screening cut point as 12.5 ng/mL of positive control antibody, see Figure 1. Due to the qualitative nature of ADA assays the sensitivity is not an absolute concentration of anti-Herceptin antibody, and may be different in a clinical setting. Use of surrogate positive control is, however, best industry practice and accepted by regulatory agencies.

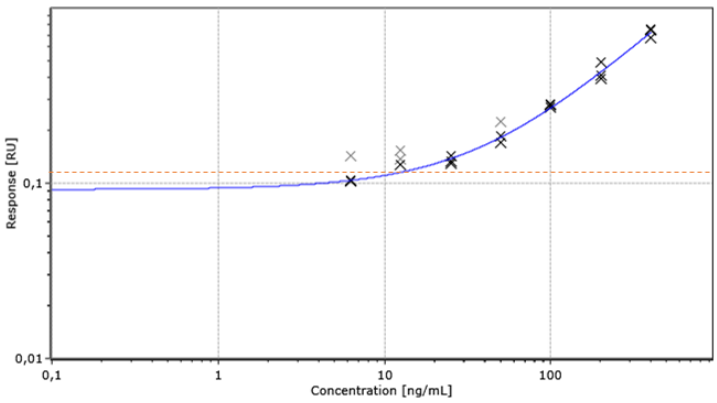


Figure 1 Positive control dilution in Rexxip ADA with 10% serum. Screening cut point 0.111 RU. Concentrations in neat serum

Assay Screening Cut Point

A floating cut point with 5% false-positive rate was estimated from 54 healthy mixed gender individual human serum samples, analyzed over 3 runs. The normalized responses (Mean Individual RU / Assay Mean NC RU) of the individuals were found to be normally distributed (Shapiro-Wilkes α=0.05) and was therefore not log transformed. Individuals 50 and 51 were found to be outliers by Grubbs test and excluded from the cut point calculations. 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 RU + 0.016 RU.

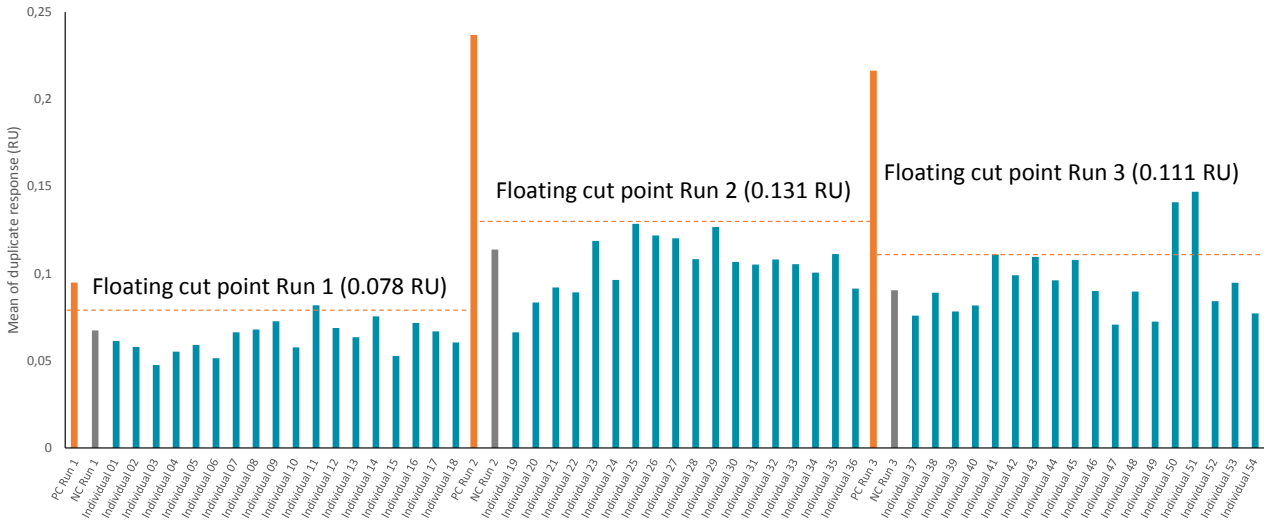


Figure 2 Average of duplicate responses (RU) from Negative Control (NC) Positive Controls (PC) at 100 ng/mL and individual human serum samples (Individual 01 – 54) in Rexxip ADA, for a final concentration of 10% serum. Concentrations of PC in neat serum.

Screening assay precision

The assay precision across three runs for the positive control at 100 ng/mL was 37% and precision for the pooled negative control over the same three runs was 23%, as seen in Table 1.

Table 1 Precision assessment of the screening assay over three runs

Parameter	Negative Control (Pooled human serum)	Positive Control (100 ng/mL)
Average response	0.0924 RU	0.185 RU
Intra-assay precision	≤17%	≤16%
Inter-assay precision	23%	37%

Confirmatory assay

54 healthy mixed gender individual human serum samples were analyzed over 3 runs following a 1-in-10 MRD using assay diluent with 200 µg/mL Herceptin and without Herceptin 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 42.1% in the presence of Rexxip ADA with 200 µg/mL Herceptin. None of the individuals that screened positive were confirmed positive.

Drug tolerance

Drug tolerance was assessed by serially diluting Herceptin 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 as 80 µg/mL of Herceptin 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 640 µg/mL of Herceptin, equivalent to average response of assay blank at 0.0944 RU; inhibition results are shown in Table 2. The anticipated trough level of Herceptin in human serum is ≤ 50 µg/mL.

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

Herceptin Conc. (µg/mL)	100 ng/mL Positive Control	
	Average response (RU)	% Inhibition
0	0.231	N/A
10	0.193	28
20	0.164	49
40	0.149	60
80	0.145	63
160	0.107	91
320	0.106	92
640	0.0793	111
1 280	0.0921	102

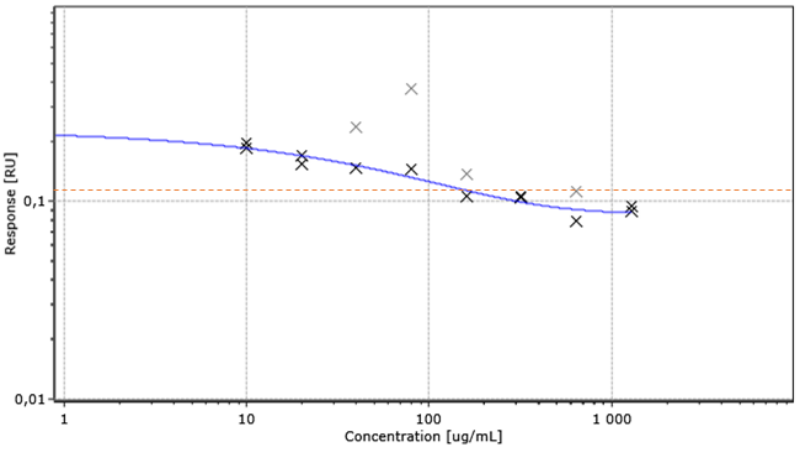


Figure 3 Dilution of Herceptin in Rexxip ADA with 10% serum and 100 ng/mL of positive control. Concentrations in neat serum.

MATERIALS AND METHODS

The assay was developed on a Gyrolab xPand system using Gyrolab Mixing CD. 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 confirmatory buffer was Rexxip ADA with 10% human serum and 200 µg/mL of Herceptin. The acid dissociation step was performed using 0.5 M Glycine-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.

Herceptin from Roche was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide), with a 10:1 challenge ratio of biotin. Capture reagent was used in Master Mix at a concentration of 12 µg/mL.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was Herceptin from Roche, diluted to a concentration of 12 µg/mL.

The positive control was a human anti-trastuzumab monoclonal antibody sourced from Bio-Rad, diluted in Rexxip ADA with 10% human serum.

Summary table

Capture	Biotin labeled trastuzumab (Herceptin, Roche), 10:1 molar challenge ratio
Detection	Alexa Fluor 647 labeled trastuzumab (Herceptin, Roche)
Master Mix	12 µg/mL detection reagent and 12 µg/mL capture reagent in 1 M Tris-HCL, pH 8
Acidic Buffer	0.5 M Glycine-HCl, pH 2.6
Analyte	Human anti Trastuzumab monoclonal antibody (HCA176; 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	23%
Suggested Positive Control concentration	PC: 12.5 ng/mL**
Confirmatory drug concentration in Rexxip ADA with 10% human serum	200 µg/mL
Assay sensitivity	12.5 ng/mL*
Drug tolerance	80 µg/mL*
Screening Cut point	0.016 RU + Mean NC RU*
Confirmatory Cut point	42.1% inhibition*

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

** Recommended Positive Control concentration updated following sensitivity assessment.

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