GYROS PROTEIN Technologies

Gyrolab® Assays

MabThera® (rituximab) PK Assay

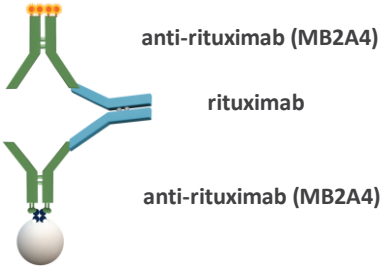
INTRODUCTION

MabThera® (rituximab) is a chimeric mouse/human monoclonal antibody. It comprises a human IgG1 constant region and murine heavy and light chain variable regions. Rituximab targets the CD20 cell surface receptor, which is solely expressed on B-cells. Upon binding, cell death is triggered via various mechanisms. Therapeutic indications are B-cell malignancies like hematologic cancer and to a lesser extent autoimmune diseases like rheumatoid arthritis.

We have developed a three-step bridging Gyrolab PK assay to determine MabThera levels in human serum samples. A dilution factior of 10 gives a broad analytical range with an approximate LLOQ of 50 ng/mL, and ULOQ of 10 000 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 anti-rituximab as capture molecule and an Alexa Fluor 647® lableled anti-rituximab detection molecule.



ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 3-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 50 ng/mL to 10 000 ng/mL (Table 1). The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in duplicate in 3 runs, was <20% (Table 2).

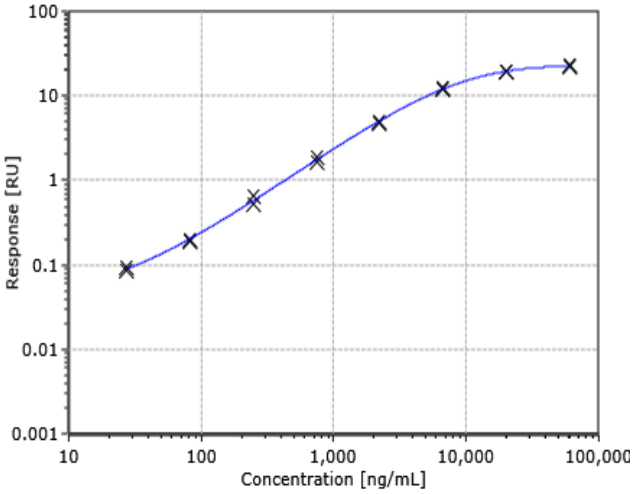


Figure 1 Standard curve in 10 % human serum. Concentrations in neat serum

Table 1 Estimated Assay Range in neat human serum, based onthree runs

LLOQ (ng/mL)	ULOQ (ng/mL)
~50	~10 000

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

QC	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)
LLOQ	50	53	13	14	23
LQC	100	119	5.0	4.4	22
MQC	1 000	1 066	14	4.5	17
HQC	3 000	3 356	5.5	3.8	15
ULOQ	10 000	11 442	4.8	5.3	20

SELECTIVITY

Selectivity was established by spiking 100 ng/mL of the drug in individual human serum samples and pooled serum. All samples measured <LLOQ when analyzed unspiked.

Table 3 Selectivity, spiked samples

Sample	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	CV%	Average Bias%
1	100	97	14	-3.2
2	100	83	11	-17
3	100	100	0.64	-0.46
4	100	104	11	4.2
5	100	103	7.7	3.3
6	100	96	4.6	-4.0
7	100	92	21	-7.8
8	100	115	1.5	-15
9	100	82	2.5	-18
10	100	67	11	-33
pool	100	99	4.7	-0.51

MATERIALS AND METHODS

The assay was developed on Gyrolab xPlore and xP using Gyrolab Bioaffy 200 CDs. The assay was set up using a 3-step method; 200-3W-002-A and the 1% PMT setting. Needle wash buffers were PBS-T (wash 1) and Gyrolab wash buffer pH11 (wash 2). The assay buffer was Rexxip H with a dilution factor of 10.

Anti-rituximab (rat IgG2a, clone MB2A4 from Bio-Rad) was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide). The storage buffer of the antibody was exchanged to PBS beforehand, using a desalting spin column (ZEBA™ spin desalting column, 7K MWCO) to ensure a buffer compatible with NHS labeling chemistry. Biotinylated BSA was purchased from Vector Laboratories (Cat. B-2007).

The capture antibody was first diluted to 100 µg/mL (700 nM) in PBS-T. Then, it was mixed with biotinylated BSA in PBS-T. Final concentrations were 10 µg/mL biotinylated anti-rituximab + 90 µg/mL biotinylated BSA in PBS-T.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the same as the capture: anti-rituximab (clone MB2A4 from Bio-Rad). The detection reagent was diluted to 25 nM in Rexxip F. The assay standard used was MabThera (rituximab) from Roche. The standard was prepared in human serum diluted 1:10 in Rexxip H.

Summary table

Capture	10 µg/mL biotinylated anti-rituximab (clone MB2A4, Bio-Rad) + 90 µg/mL biotinylated BSA in PBS-T
Detection	Alexa Fluor 647 labeled anti-rituximab (clone MB2A4 Bio-Rad), 25 nM in Rexxip F
Analyte	Rituximab (Roche) in Rexxip H with 10% human serum (higher % serum possible)
CD-type	Bioaffy 200 CD (Bioaffy 1000 CD if better sensitivity is required)
Method	200-3W-002-A (or 1000-3W-006-A for Bioaffy 1000 CD)
Wash buffer for needles	Wash buffer 1: PBS-T, wash buffer 2: Gyrolab wash buffer pH 11
PMT-setting	1% PMT
Expected dynamic range	Approximate 50 – 10 000 ng/mL in neat serum (dilution factior 10, less dilution possible)

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

If a higher sensitivity is required, this assay could be run with a Bioaffy 1000 CD using a similar method (1000-3W-006-A). The higher sample volume will increase the sensitivity. Furthermore, the dilution factor can be decreased, as there were no matrix effects observed at dilution factor 10.

For additional support contact your local Field Application Support