Ultra Sensitive PK Bioanalysis in Support of the Development of a Bispecific Immune-Recruiting Biotherapeutic

chimera biotec
ultra sensitive immunoassays

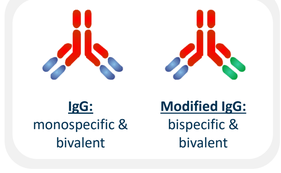
Christian Pieper, Michael Adler, Bianca Brüggen and Mark Spengler

INTRODUCTION

BACKGROUND

Bispecific antibodies recruiting T-cells are used as biotherapeutic drugs. Their excellent specificity and selectivity in combination with their ability to recruit immune-effector cells to tumor tissue makes them very attractive candidates in fighting cancer.

However, T-cell-based therapies have been associated with TOX profiles (e.g. potential cytokine release syndrome) which need to be carefully managed.
Based on their high potency, initial dosing in clinical trials is very low. In consequence, the expected drug concentrations in PK profiling are below technical limitations of conventional ELISA-type ligand-binding assays (LBAs).
Thus, novel assays are required to overcome these limitations.

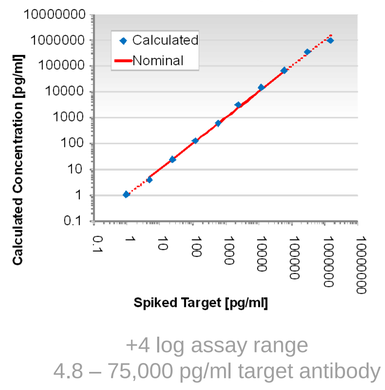


METHOD

Imperacer® Immuno-PCR assay development and bioanalytical method validation in support of clinical trials, utilizing ultra sensitive tailored antibody-DNA detector conjugates.

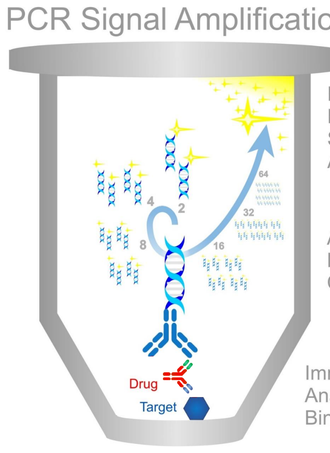
METHOD

Validated Standards & QCs



+4 log assay range
4.8 – 75,000 pg/ml target antibody

PCR Signal Amplification

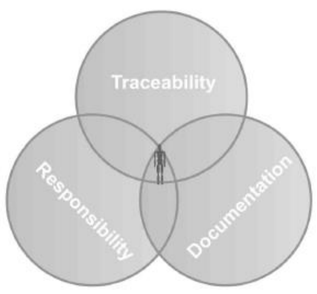


Exponential PCR Signal Amplification

Antibody-DNA Detection Conjugate

Immunoassay Analyte Binding

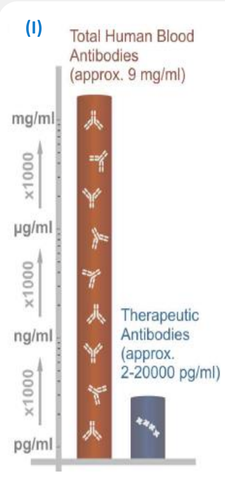
GCP

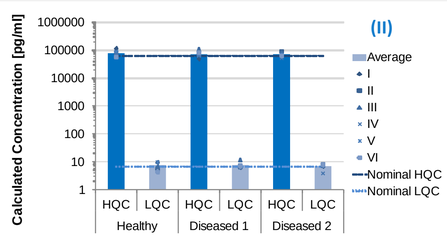


- Expertise in assay development and application
- In-house quality monitored preparation of key reagents
- Sample stability and reagent handling in sub-µl up to ml range
- 24/7 monitored sample storage facilities
- Tailored protocols for ultra sensitive data analysis

Analysis in accordance to GCP/GLP is mandatory for regulatory-conform data generation which transcend research evaluations. The application of GCP and GLP requirements to ultra sensitive analytics is a novel challenge which becomes more important with the advent of new technologies.
The Imperacer® assay as well as the laboratory facilities fulfill all requirements for GCP-conform analysis as appropriate for human sample material from clinical studies.

SELECTIVITY





1+19 Dilution
4 µl sample
+ 76 µl SDB

Chimera´s ***AnySource®*** sample dilution in tailored **S**ample **D**ilution **B**uffer (**SDB**) minimizes matrix effects, thereby enabling therapeutic antibody target detection
(I) in the presence of approx. **1,000,000-fold excess** of endogenous antibodies (human IgG, IgA, IgM, etc.)
(II) in matrices from different hematologic patient sub-populations.

Therapeutic antibody drug was spiked into serum from healthy individuals and two different diseased patient sub-populations. Quantification with a calibration curve in an in-house prepared qualified standardized serum pool revealed passed acceptance criteria for all matrices. Therefore, the same analytical protocol can be applied to the different hematologic patient sub-populations.

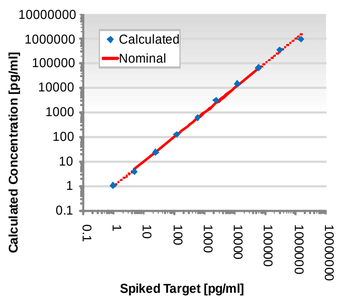
VALIDATION

Nominal [pg/ml]	S1 (ULOQ) 75000	S2 15000	S3 3000	S4 600	S5 120	S6 24	S7 (LLOQ) 4.8	S8 (Anchor) 0.96	HQC 62500	MQC 500	LQC 8
Accuracy											
N	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	12.0	12.0	12.0
Inter-Assay Mean	77406.3	14568.4	2894.2	617.2	126.4	23.81	3.97	1.070	66929.90	503.40	8.55
%RE	3.2	2.9	3.5	2.9	5.3	0.8	17.2	11.4	7.1	0.7	6.8
Intra-Assay Range of %RE	0.1 - 6.4	-7.9 - 2.2	-8.4 - 2.3	-1.8 - 11.1	-0.7 - 8.4	-7.2 - 4.1	-49.4 - 0.4	-0.1 - 17.6	-5.71 - 29.13	-6.01 - 5.42	1.04 - 14.69
RE%< 20% (25% at ULOQ/LLOQ)	100%	100%	100%	100%	100%	100%	83.3%	100%	83.3%	100%	100%
Intra-assay precision											
Range of %CV	0 - 13.6	1 - 5.9	0.6 - 8.6	1.2 - 13.1	0.3 - 8.5	2.8 - 14.3	1 - 26.8	2.9 - 41.1	0.24 - 14.50	0.35 - 23.61	0.63 - 17.87
CV%< 20% (25% at ULOQ/LLOQ)	100%	100%	100%	100%	100%	100%	83.3%	50%	100%	100%	100%
%CVp (de Silva)									6.4	9.7	7.7
Inter-assay precision											
SDbetween means	1853.4	520.5	104.3	27.0	4.35	0.97	0.80	0.065	7807.75	26.70	0.38
%CV	2.4	3.6	3.6	4.4	3.4	4.1	20.2	6.0	12.5	5.3	4.7
%CVt (de Silva)									12.5	9.7	7.7
%Total Error	*5.6*	*6.5*	*7.1*	*7.2*	*8.7*	*4.9*	*37.4*	*17.5*	*19.6*	*10.4*	*14.5*

Imperacer® was challenged with inter-assay 6-fold determination of standards (duplicates) and QCs (2-fold duplicates). Additional tests included dilution linearity, robustness against variation of Imperacer® detection instruments, application specialists and different lots of detection conjugate, selectivity as well as testing for freeze-thaw, short- & long-term stability.

Both, drug stability and analytical technology revealed passed acceptance criteria (typical limits: 20-25% for accuracy and precision), confirming compatibility with regulatory requirements and thereby enabling the method for GCP applications.

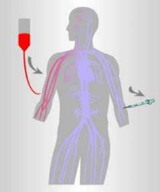
SENSITIVITY



Maximum sensitivity:
0.96 pg/ml non-diluted sample (Anchor) in 30 µl assay volume, 1+19 dilution:
1.44 fg/well

Maximum range
>1 µg/ml (confirmed in dilution linearity)

From therapy to sample



High dosing:
Approx. 100 µg drug
Max. approx.
20 ng/ml in blood

Low dosing:
Approx. 10 ng drug
Max. approx.
2 pg/ml in blood

Drug application determines meaningful required detection range:
While dose-escalation studies start at low dosing, higher dosing* as well as toxicokinetic evaluations may require additional assay compatibility with >10,000-fold higher concentrations.
A broad dynamic assay range enables evaluation of drug levels with a single assay protocol and can further be enhanced with additional sample dilution steps.
Absolute sensitivity in the assay is determined by drug concentration & dilution.

* T-cell engager start with low doses independent of format, see e.g. Yuraszeck *et al.,* 2017, *Clin Pharmacol*, 101, 634.

SUMMARY

Results

An Imperacer® assay for a bispecific antibody was developed with +4 log assay range from 5 pg/ml – 75,000 pg/ml and fully validated according to bioanalytical method validation guidance documents. A special focus was laid on matrix adaptation via Chimera´s AnySource® sample dilution technology. GCP bioanalyses to support clinical studies are ongoing with excellent assay performance in different hematologic patient sub-populations.

Conclusion

Here we present assay development and method validation of an ultra sensitive PK assay for GCP regulated bioanalytical sample testing support of a T-cell recruiting bispecific antibody in development for the treatment of hematological malignancies. The provided combination of broad assay range and high sensitivity is a powerful tool in PK analysis to quantify low dosed drug concentrations.