Comparison of Non-target Specific, Mono- and Bi-functional PK Assays Formats Targeting a Bispecific mAb

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Introduction

The therapeutic mAb considered here is a bispecific mAb developed for the treatment of inflammatory diseases by targeting two different cytokines. Several approaches were used to detect the compound in human serum by using either:

- a monoclonal anti-idiotypic antibody for capture and detection (non-target specific format),
- a monoclonal anti-idiotypic antibody for capture and one of the targeted cytokines for detection (mono-functional format),
- both targeted cytokines for capture and detection (bi-functional format).

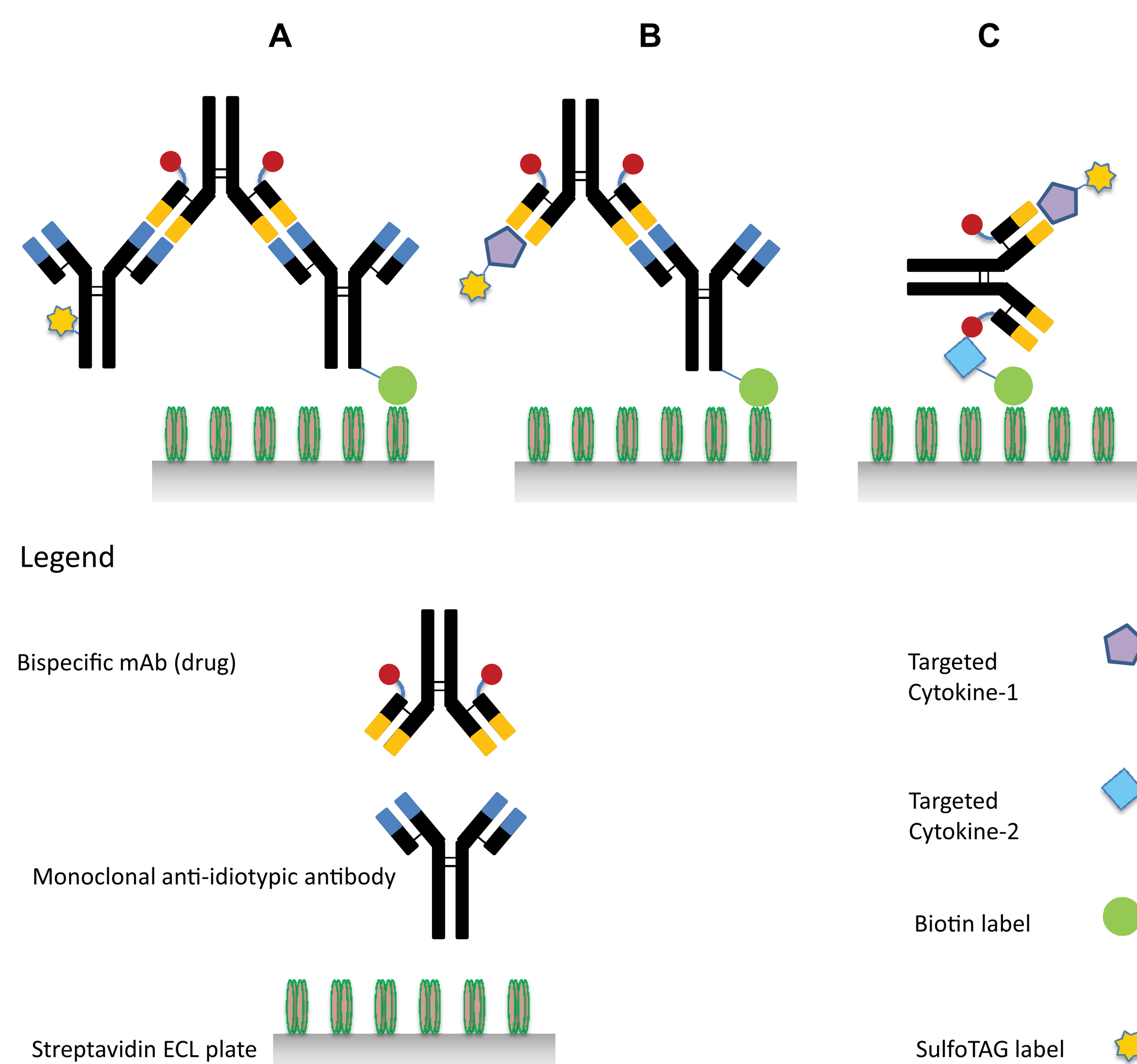
The non-target specific format was developed to confirm data obtained with the mono- and bi-functional assays. For this assay, samples were pre-treated to dissociate immune complexes formed between the drug and circulating cytokines and/or anti-drug antibodies (ADA).

Each format was optimized independently for protein labeling, minimum required dilution, conditions of incubations as well as concentration of key reagents. Moreover, interference with circulating cytokines was assessed as part of method qualifications. Clinical samples were measured with the three different formats. Intravascular and subcutaneous routes of administration were considered. Results from three representative subjects from each group were compared and pharmacokinetic (PK) profiles further analyzed in light of corresponding ADA profiles.

Analytical Methods

Non-target specific, mono- and bi-functional PK assay formats are presented in Figures 1A, 1B and 1C respectively. Immune complexes were detected by electrochemiluminescence using a Sector Imager 2400 (Meso Scale Discovery).

Figure 1: Non-target Specific (A), Mono- (B) and Bi-functional (C) PK Assays



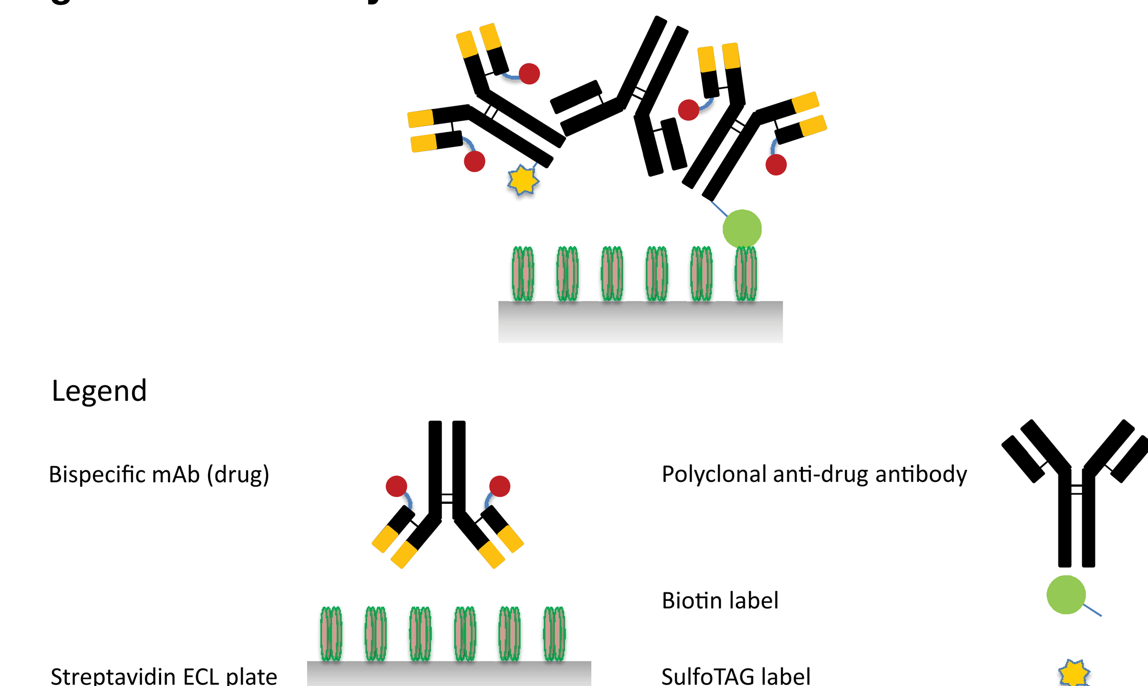
A comparison of PK assay format performances is shown in Figure 2. Pre-treatment led to a slightly higher dilution of samples and therefore a higher LLOQ with the non-target specific format.

Figure 2: Comparison of PK Assays

	Assay Format		
	Non-target specific	Mono-functional	Bi-functional
Analytical Range (ng/mL)	20.0 - 3200	10.0 - 4000	10.0 - 4000
Sample Dilution	15	5	5
Sample Volume (µL)	20.0	20.0	20.0

In addition to PK assessments, ADA was detected using a sandwich format, with labeled drug for capture and detection (Figure 3). Screening, confirmatory and titer assays were setup and optimized for free drug tolerance.

Figure 3: ADA Assay



Results

Tolerances to circulating cytokines assessed with the non-target specific, mono- and bi-functional PK assays are presented in Figures 4A, 4B and 4C respectively.

Measured concentrations with the non-target specific, mono- and bi-functional formats were similar between formats. Representative PK profiles for the intravenous and subcutaneous cohorts are shown in Figures 5A and 5B, respectively.

Correlations between non-target specific and mono-functional, non-target specific and bi-functional, mono- and bi-functional PK assays are shown in Figures 6A, 6B and 6C respectively. Measured drug concentrations showed high correlations between formats (r^2=0.9638, n=54; r^2=0.9385, n=52 and r^2=0.984, n=52 for correlations between non-target specific and mono-functional, non-target-specific and bi-functional, mono- and bi-functional assays respectively). Even higher correlations were observed in subjects treated subcutaneously. Within this cohort, the range of measured concentrations was narrower (14.2-4540 and 21.3-20700 ng/mL for subcutaneous and intravenous routes, respectively).

PK measurements at late time points (days 8-29) are presented in Figures 7A and 7B, for the intravenous and the subcutaneous cohorts respectively. Example of PK and ADA titer assessments through time are presented in Figure 8.

Figure 4: Tolerances to Circulating Cytokines Assessed with the Non-target Specific (A), Mono- (B) and Bi-functional (C) PK Assays

A.
Non-target Specific Assay: Interference with Cytokine-1 and Cytokine-2

	Conc. Cytokine-1 and Cytokine-2 (ng/ml)							
	Blank	0.005	0.05	0.5	5	50	500	5000
Drug Theor. Conc. (ng/mL)	200	200	200	200	200	200	200	200
Found Conc. (%Theoretical)	99.7	101	101	105	107	107	106	85.6

B.
Mono-Functional Assay: Interference with Cytokine-1

	Conc. Cytokine-1 (pg/mL)							
	Blank	0.032	0.16	0.8	4.0	20	100	500
Drug Theor. Conc. (ng/mL)	10	10	10	10	10	10	10	10
Found Conc. (%Theoretical)	117	104	100	105	109	88.8	90.9	91.3

C.
Bi-Functional Assay: Interference with Cytokine-1

	Conc. Cytokine-1 (pg/mL)							
	Blank	0.032	0.16	0.8	4.0	20	100	500
Drug Theor. Conc. (ng/mL)	10	10	10	10	10	10	10	10
Found Conc. (%Theoretical)	107	103	105	103	109	101	91.6	99.0

Bi-Functional Assay: Interference with Cytokine-2

	Conc. Cytokine-2 (pg/mL)							
	Blank	0.032	0.16	0.8	4.0	20	100	500
Drug Theor. Conc. (ng/mL)	10	10	10	10	10	10	10	10
Found Conc. (%Theoretical)	96.6	104	103	105	104	103	89.2	95.3

Figure 5: Representative PK Profiles from Intravenous (A) and Subcutaneous Cohorts (B) When Assessed with the Non-target Specific, Mono- and Bi-functional PK Assays

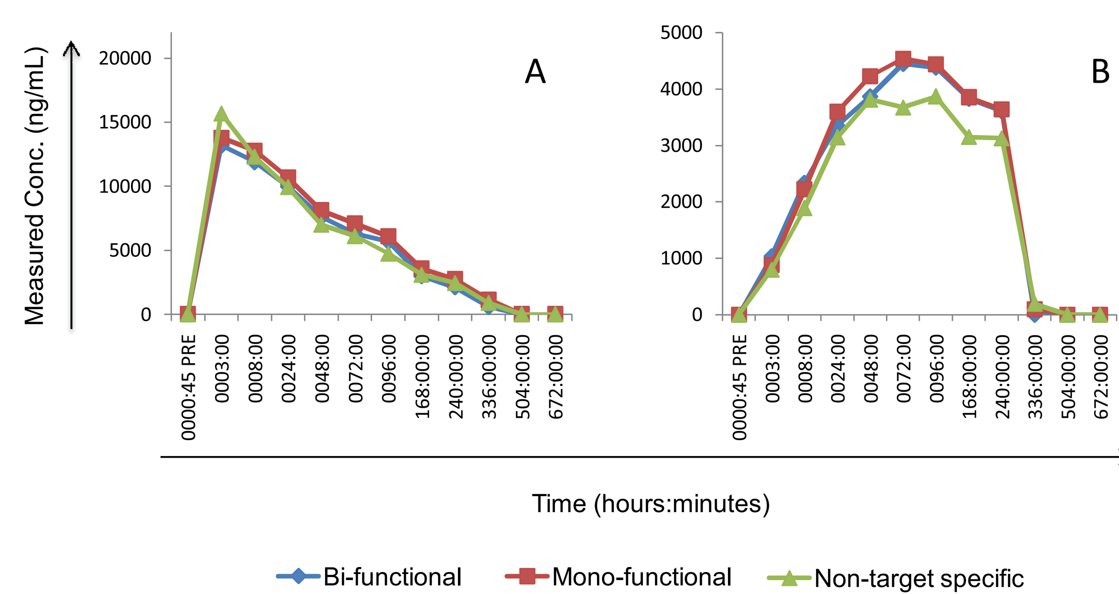


Figure 6: Correlations between Non-target Specific and Mono-functional (A), Non-target Specific and Bi-functional (B), Mono- and Bi-functional (C) PK Assays

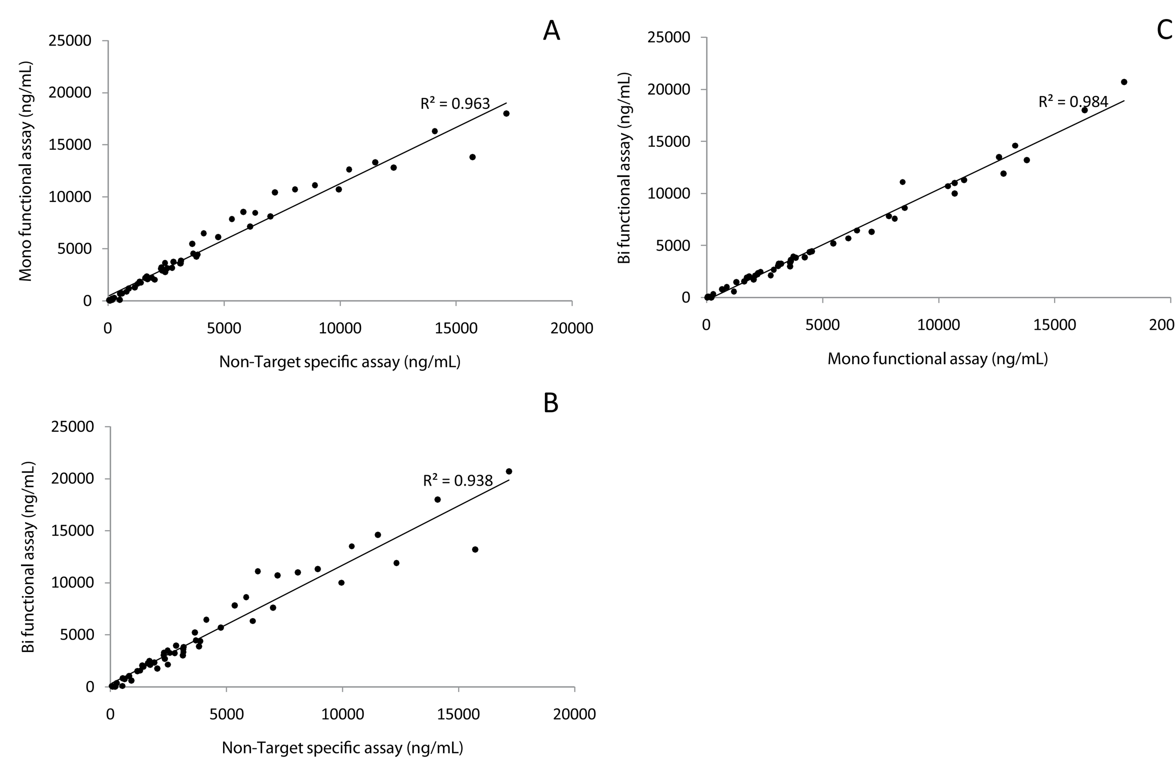


Figure 7: PK Assessments in Intravenous (A), and Subcutaneous (B) Cohorts at Late Time Points

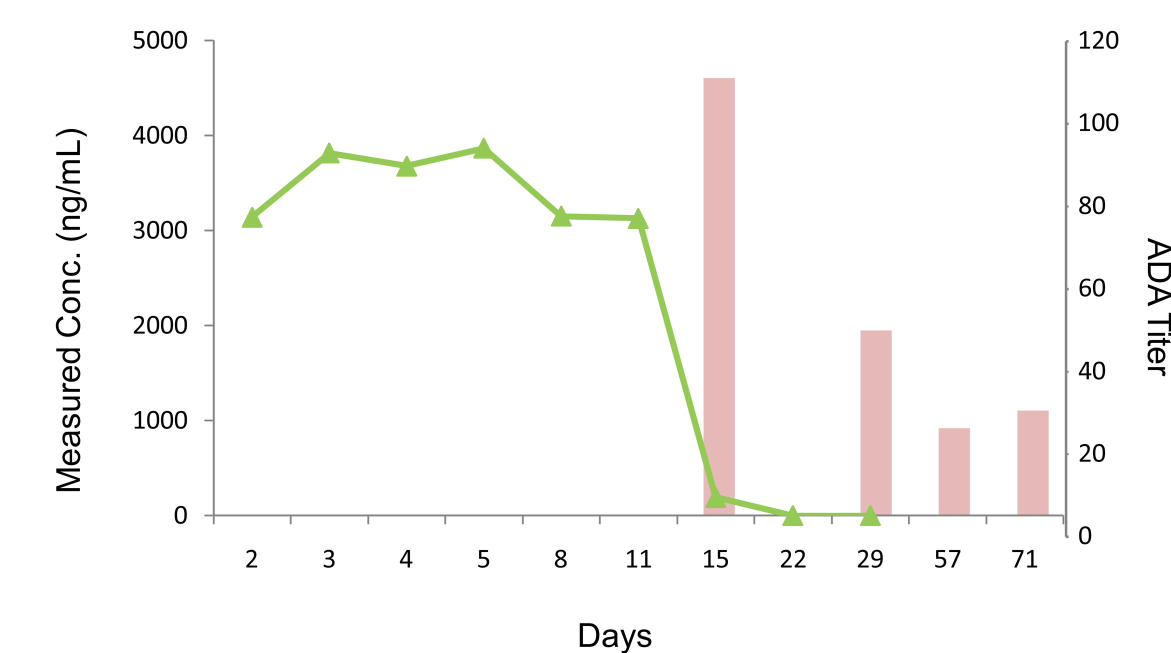
A.

		Intravenous Drug (ng/mL)		
Subject ID	Day	Non-target specific	Mono-functional	Bi-functional
1	8	3119	3600	3010
1	11	2474	2770	2130
1	15	901	1180	597
1	22	<20.0	<10.0	<10.0
1	29	<20.0	<10.0	<10.0
2	8	2462	3640	3490
2	11	2343	2900	2680
2	15	207	213	24.5
2	22	<20.0	<10.0	<10.0
2	29	<20.0	<10.0	<10.0
3	8	2013	2030	1750
3	11	512	108	71.9
3	15	209	<10.0	<10.0
3	22	152	<10.0	<10.0
3	29	88.3	30.7	21.3

B.

		Subcutaneous Drug (ng/mL)		
Subject ID	Day	Non-target specific	Mono-functional	Bi-functional
4	8	3149	3850	3830
4	11	3128	3640	3610
4	15	191	94.7	<10.0
4	22	<20.0	<10.0	<10.0
4	29	<20.0	<10.0	<10.0
5	8	2312	3210	3260
5	11	2285	3090	3030
5	15	85.3	37	<10.0
5	22	<20.0	<10.0	<10.0
5	29	<20.0	<10.0	<10.0
6	8	1605	2190	2250
6	11	1709	2090	2090
6	15	192	204	14.2
6	22	<20.0	<10.0	<10.0
6	29	<20.0	<10.0	<10.0

Figure 8: Non-target specific PK and ADA titer profiles observed in a subject from the subcutaneous cohort



Discussion

In the present work, three formats of PK assays were developed to measure the concentration of a therapeutic bispecific mAb in human serum.

- Assay formats sharing key reagents –either for capture or for detection- showed highest correlations regarding the measured concentrations of the drug.
- Pre-treatment of samples applied in the non-target specific format led to accurate drug detection in presence of high cytokines concentrations.
- At the latest time points, the dissociation of drug-ADA complexes in samples pre-treated for the non-target specific assay led to higher found concentrations in comparison with the other formats.
- Decreasing circulating drug concentrations measured at the latest time points were associated with increasing ADA titers.

Conclusions

The PK assays described here showed improved reliability, maximal reproducibility and robustness. Associated to ADA and isotyping assays developed at Celerion Switzerland AG, they offer important tools for early clinical development of bispecific antibodies.

Poster presentation at EBF 2016

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