Immunogenicity Assessment By RIA, Not Just A Method Past Its Prime

S. Fleire, P. Doytcheva, K. Sklodowski, P. Brennecke and P. Struwe
Celerion Switzerland AG, 8320 Fehraltorf, Switzerland

Introduction

Over the course of treatment with biologics, patients may develop anti-drug antibodies that could impair the "functionality" of the drug (PK performance), as well as trigger serious hypersensitivity reactions. Therefore, monitoring of anti-drug antibodies (ADA) is key to evaluate the safety of biologics during clinical trials and post-market surveillance.

Radioimmunoassays (RIA) remain a highly sensitive and robust bioanalytical method in immunogenicity assessments, particularly for peptides.

In the present study, we developed and validated a state-of-the-art RIA assay for the detection of ADA raised against a blood coagulation component, regularly administered to haemophilic patients.

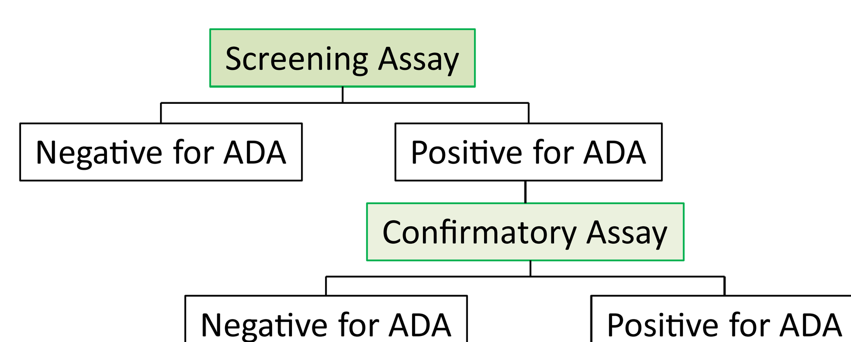
The assay showed a sensitivity of 15 ng/mL (screening assay) with a sample volume of 20 µL (requirement for pediatric patients) and was inert to matrix effects.

In conclusion, we developed a very robust RIA assay for the detection of ADAs raised against a drug administered blood clotting factor. Proper assessment of this kind of antibodies is crucial for closely monitoring the potentially deleterious effects of drug "failure".

Analytical Methods

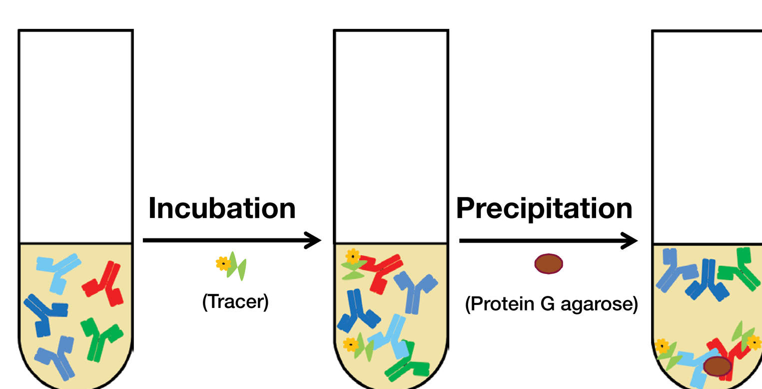
Analysis of immunogenicity follows a tiered approach according to the commonly applied guidelines (US FDA 2016) (Figure 1). Therefore, a screening and a confirmatory assay were developed.

Figure 1: Tiered approach for Immunogenicity assessment



The assay uses the radiolabeled drug (tracer) in a two-step method to detect ADAs. First, plasma samples are incubated overnight with the tracer to allow for binding to ADAs; second, antibodies are captured and precipitated with protein G agarose beads (Figure 2). In the confirmatory format, an excess of non- radioactive drug is added during overnight incubation to compete the tracer out.

Figure 2: Assay Format



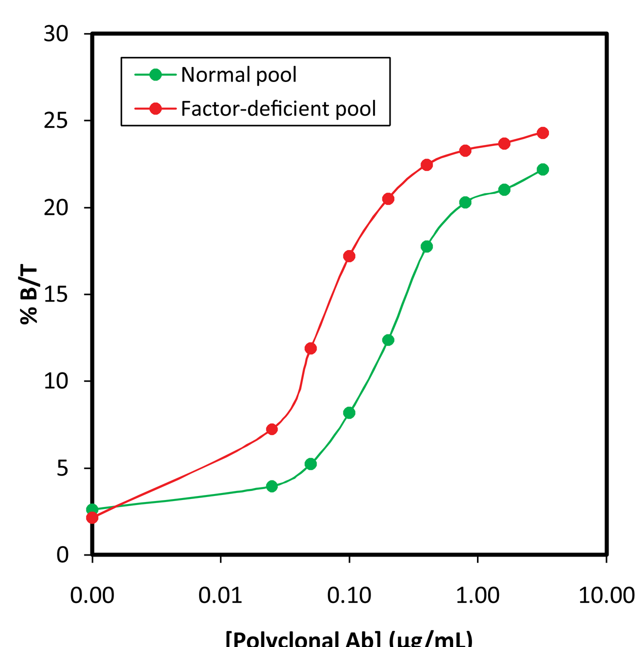
The radioactivity associated with the precipitated ADAs is proportional to the concentration of anti-factor antibodies in the sample.

Analytical Development

1: Matrix Interference

Due to the presence of the endogenous coagulation factor, matrix interference was expected and evaluated by comparing dilution curves of the positive control antibodies (polyclonal anti-drug antibodies) prepared in normal and in factor-deficient human plasma pool (Figure 3).

Figure 3: Matrix Interference Evaluation



The endogenous factor proved to have a negative effect on the dose-response curve, but not on the background (no antibody added). Therefore, for further development, factor-deficient plasma pool was employed.

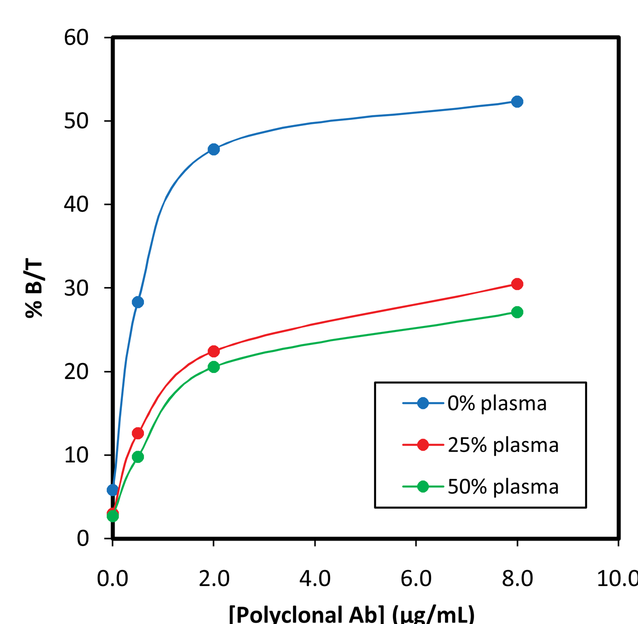
2: Minimum Required Dilution Assessment

In order to better characterize and minimize the impact of the matrix effect on the assay, two experiments were performed:

- assessment of minimum required dilution (MRD)
- heterogeneity of individual samples

To assess the MRD, dose-response curves prepared in buffer with different concentration of plasma pool were compared (Figure 4).

Figure 4: Minimum Required Dilution Assessment



A significant difference in binding was observed between the dose-response curve prepared in buffer (0 % plasma) and the dose-response curves prepared in buffer containing 25 or 50 % (MRDs 1:17.5 and 1:7, respectively), confirming the matrix effect observed. However, no significant difference in sensitivity was observed between 25 or 50 % plasma-containing samples.

To test the heterogeneity of the individuals, six diseased population plasma samples were also spiked with varying concentrations of the polyclonal anti-factor antibody and analyzed at two different MRDs (Table 1).

Table 1: Heterogeneity of Individual Samples

Individual	[Polyclonal Ab] (µg/mL) MRD 1:17.5				MRD 1:7			
	0	0.5	2	8	0	0.5	2	8
1	2.92	5.70	13.69	25.41	3.11	8.17	16.88	28.11
2	2.99	6.56	13.24	24.62	2.46	9.20	18.86	23.91
3	3.20	6.57	13.82	24.11	3.67	10.22	21.24	26.38
4	3.10	6.17	12.13	23.93	2.40	8.05	18.43	23.56
5	2.23	5.20	11.33	20.32	2.56	7.99	17.21	21.68
6	2.76	6.14	12.74	24.19	2.76	8.55	17.08	24.13
Mean	**2.87**	**6.06**	**12.83**	**23.76**	**2.83**	**8.70**	**18.28**	**24.63**
SD	0.35	0.53	0.96	1.77	0.49	0.87	1.65	2.27

No significant differences were observed between the individuals or between the two MRDs, indicating that both were appropriate to further develop the assay.

Due to the requirement of the assay to be adapted to low sample volumes (pediatric patients), the highest MRD (1:17.5) was selected.

Assay Validation

The optimized assay was validated following international standards. The following parameters were evaluated:

- screening cut point estimation
- specificity cut point estimation
- sensitivity of the screening assay
- sensitivity of the confirmatory assay
- precision
- selectivity/recovery
- hook effect
- free drug tolerance
- stability

Only critical parameters are presented.

Based on the results obtained during method development, quality control samples (QCs) were prepared in factor-deficient plasma pool, and cut-point determination was evaluated with 50 healthy individual samples (did not show significant differences in the unspiked samples, see Figure 1).

The QC levels were defined as:

QC_0: 0 µg/mL
QC_{low}: 0.6 µg/mL
QC_{med}: 5 µg/mL
QC_{high}: 18 µg/mL

1: Precision

Precision was evaluated in 6 analytical runs (inter-run precision), and six duplicates of each control level (intra-run precision).

	% CV QC_0	QC_{low}	QC_{med}	QC_{high}
Inter-assay	25.4	14.7	11.1	11.4
Intra-assay	11.1	7.2	5.7	4.4

2: Sensitivity

Sensitivity for the screening and confirmatory formats was evaluated in six runs, with two independent curves prepared in each run.

Screening format:

	Interpolated conc. at cut-point [µg/mL]	Log(Interpolated conc. at cut-point)
Run 1	0.35	-0.458
	0.34	-0.464
Run 2	0.28	-0.548
	0.24	-0.625
Run 3	0.37	-0.431
	0.20	-0.689
Run 4	0.13	-0.884
	0.27	-0.572
Run 5	0.34	-0.463
	0.29	-0.540
Run 6	0.26	-0.583
	0.19	-0.726
Mean [ng/mL]		-0.582
SD [ng/mL]		0.133
n		12
Log screening sensitivity limit at 99%		-0.221
Log screening sensitivity limit at 95%		-0.343
Screening sensitivity limit at 99% [ng/mL]		0.602
Screening sensitivity limit 95% [ng/mL]		0.454

*Based on a content of anti-factor specific antibodies in the polyclonal serum of 2.5 %, the sensitivities for the two confidence intervals are 15.04 ng/mL and 11.34 ng/mL (99 % and 95 % confidence, respectively).

Confirmatory format:

	Interpolated conc. at cut-point [µg/mL]	Log(Interpolated conc. at cut-point)
Run 1	0.31	-0.515
	0.23	-0.642
Run 2	0.35	-0.451
	Invalid	N/AP
Run 3	0.26	-0.584
	Invalid	N/AP
Run 4	0.14	-0.861
	0.31	-0.502
Run 5	0.16	-0.800
	0.15	-0.837
Run 6	0.49	-0.309
	0.50	-0.299
Mean [ng/mL]		-0.580
SD [ng/mL]		0.205
n		10
Log screening sensitivity limit at 99%		-0.003
Log screening sensitivity limit at 95%		-0.205
Screening sensitivity limit at 99% [ng/mL]		0.994
Screening sensitivity limit 95% [ng/mL]		0.624

*Based on a content of anti-factor specific antibodies in the polyclonal serum of 2.5 %, the sensitivities for the two confidence intervals are 24.85 ng/mL and 15.6 ng/mL (99 % and 95 % confidence, respectively).

3: Recovery

Recovery (selectivity) was evaluated with ten individual samples (factor-deficient individuals) spiked at the QClow (low spike) and QC_{high} (high spike) levels.

	Low spike evaluation			High spike evaluation		
Matrix	Mean B/T %	CV (%)	Recovery (%)	Mean B/T %	CV (%)	Recovery (%)
1	6.16	3.30	103	28.59	1.55	100
2	6.78	6.20	113	28.87	10.83	101
3	6.02	2.85	101	27.15	2.41	95
4	6.17	5.05	103	30.06	6.67	105
5	5.34	0.17	89	24.73	1.50	86
6	5.50	1.89	92	28.42	4.72	99
7	6.06	7.08	101	30.07	8.25	105
8	7.28	8.69	122	27.11	2.18	95
9	6.13	6.05	102	27.11	2.72	95
10	5.47	2.31	91	26.46	6.23	93
Pool	5.99	3.62	-	28.59	4.50	-
Samples meeting acceptance criteria (%)			90			100

Discussion and Conclusions

During the development of a RIA to detect ADAs to a factor involved in the coagulation cascade, a strong matrix effect was detected.

With careful evaluation of MRD and proper selection of matrix (factor-deficient plasma), this interference was efficiently abrogated, as confirmed by recovery experiments.

Validation of the assay confirmed the observations obtained during assay development yielding a robust and sensitive assay, as well as requiring a low sample volume (20 µL).

Radioimmunoassay (RIA) methods are robust and reliable methods for the evaluation of immunogenicity towards peptides and are not just methods past their prime.

References

- Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products. Guidance for Industry. Draft Guidance. US FDA 2016

Poster presentation at EBF 2018