Introduction

Over the course of treatment with biologics, patients may develop anti-drug antibodies that could impair the “functionality” of the drug (e.g. 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.

Radioimmunosassays (RIA) remain a highly sensitive and robust analytical 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 radiolabeled drug (tracer) in a two-step method to detect ADAs. First, plasma samples are incubated overnight with the antigen to allow for binding to ADAs; second, antibodies are captured and detected using a second antibody.

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>
<thead>
<tr>
<th>Table 1: Heterogeneity of Individual Samples</th>
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<tr>
<td>Individual</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>5</td>
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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:

- Sensitivity
- Specificity
- Selectivity/recovery
- Free drug tolerance
- Stability
- Precision
- Linearity
- Stability
- Robustness
- Carry-over
- Recovery

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:

- QCu: 0 ng/mL
- QCu: 0.6 ng/mL
- QCu: 8 ng/mL
- QCu: 16 ng/mL

1: Precision

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

<table>
<thead>
<tr>
<th>Table 1: Precision</th>
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<tr>
<td>QCu [ng/mL]</td>
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<tr>
<td>0.00</td>
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<tr>
<td>0.60</td>
</tr>
<tr>
<td>8.00</td>
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<td>16.00</td>
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To assess the MRO, dose-response curves prepared in buffer with different concentration of plasma pool were compared (Figure 4).

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).

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

References