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Method validation approach for the determination of antibody response to biopharmaceuticals

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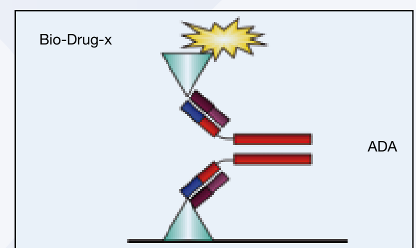
Introduction

Biopharmaceuticals are increasingly used as therapeutic agents, but may induce humoral and cellular immune responses with considerable consequences. It is therefore essential to select and/or develop assays for assessment of such immune responses. Recently, we validated a bridging ELISA format for the detection of anti-Drug-Antibodies (ADAs) against Drug-X according to the latest developments in the immunogenicity field. This ELISA-based immunogenicity test consists of 2 assays: (I) a screening assay to discriminate between positive or negative samples and, (II) a confirmation assay based on a competition ELISA to verify positivity of samples. We determined the screening assay cut-point for Drug-X with two technicians, who each analyzed 50 drug naïve serum samples. With the outliers removed we calculated the average signal of the Negative Control, the Standard Deviation and Normalization Factor, which is used to establish an assay run specific cut point with a false positive rate of 5%. Based on results obtained from 50 drug naïve serum samples, a low quality control (low positive sample) was calculated on the basis of 1% rejection rate. During method validation we additionally performed experiments that contribute to a reliable sample assessment, including Limit of Detection, precision and selectivity experiments. Furthermore, we demonstrated that anti-Drug-X antibodies are stable for 1 day at bench-top temperature, for 3 freeze-thaw cycles and 1 month at -70°C. We validated a confirmation assay, used to eliminate false positive samples/patients identified during screening of samples. In conclusion, we set up and validated a specific method to investigate immunogenicity of Drug-X in human serum, in compliance with OECD-GLP and based on the latest developments in the immunogenicity field.

Immunogenicity ELISA Assay

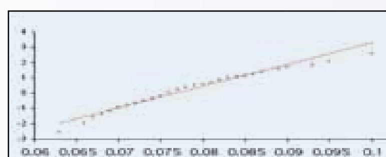
The immunogenicity ELISA assay was developed to quantify a total Ig antibody response to Drug-X in human serum. Drug-X was immobilized on 96 wells plate, blocked and diluted serum samples were incubated for 1 hour. Possible anti-Drug-X antibodies (ADAs) were bound to the Drug-X on the plate. Biotinylated Drug-X in combination with Streptavidin-HRP and TMB was used to generate a signal. Affinity purified rat-polyclonal anti-Drug-X serum was used in this assay as positive control.



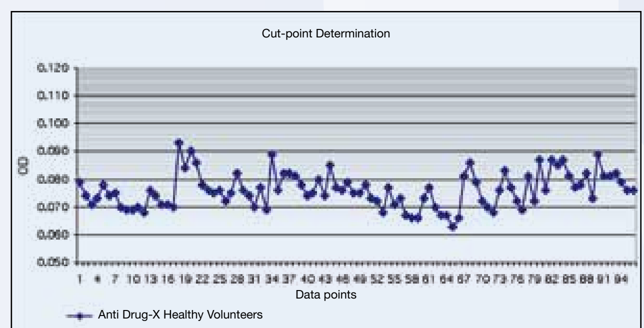
Cut-point Determination

A sample is considered positive if the response is above the cut-point. To determine the assay cut-point, two technicians analyzed each 50 individual serum samples from healthy volunteers over three different days. From this dataset outliers were removed with a Shapiro-Wilk test and the cut-point was determined statistically on a 95% upper confidence limit <1.645xSD-cut-point determination>(Normalization factor). 10 sera, analysed in the Cut-point determination, were pooled and used during sample assessment as negative control (NC). The negative control is one of the most important assay parameters for sample assessment, as the assay Cut-point is defined as: OD NC + Normalization factor (Floating Cut-point).

— 95% CI Notched Outlier Boxplot Median (0.0760)
— 95% CI Mean Diamond Mean (0.0767)
+ Outliers > 1.5 and < 3 IQR



Mean OD and normalization factor	
Mean OD	0.076
SD	0.006
Norm. factor	0.010

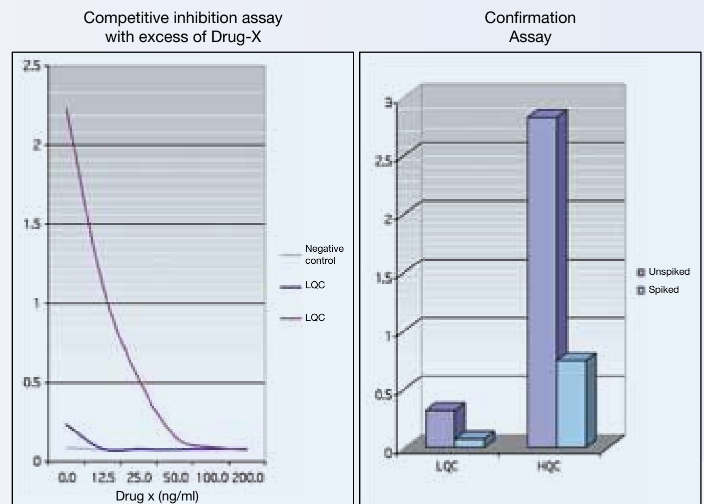


Limit of Detection

The limit of detection (LOD), is defined as the amount of analyte required to produce a significant change in signal versus that obtained in the absence of analyte. The sensitivity was determined by preparing a calibration curve by spiking a serum pool with anti-Drug-X antibodies. The LOD is the concentration at which the positive control produces a response equal to the cut-point. Back-calculating the LOD from this curve resulted in a concentration of 75 ng/mL. Secondly, a positive control that mimics a low affinity sample was established. This QC sample was defined as: Mean Cut-point + (2.33*SD Cut-point), and resulted in a concentration of 16 ng/mL.

Confirmation Assay

A Confirmation assay is essential for the elimination of false positive samples/patients following the initial screening and is based on a competitive inhibition test. Samples are incubated with an excess of drug in order to preoccupy antibody binding sites. By using this incubation step, it is possible to distinguish between true positive samples (absence of signal) and false positive samples (presence of signal).



25 ng/mL of Drug-X results in a drop of more than 50% of the signal in the confirmation assay.

Additional Experiments	
Determination Upper Acceptance Limit of the Negative control	OD: 0.101
Assay Drift	Not Detected
Precision Control Samples (LQC, HQC and negative control)	< 16.3%
Bench-Top stability	1 Day
Freeze-Thaw Stability	3 Cycles
Long term Stability QC and Samples	1 Month

Conclusion

Following validation of a method for the quantitative determination of an antibody response to biopharmaceutical, Drug-X, it was demonstrated that the method was precise with an overall precision of less than 16 %. The LOD determined to be 75.5 ng/mL. An assay-specific cut-point for total Ig in healthy volunteers was established. Stability of anti-Drug-X antibodies in human serum was demonstrated for at least 1 day at bench-top temperature, 1 month at –70°C and 3 freeze/thaw cycles. To confirm specificity of positive Anti-Drug-X samples a confirmation assay was validated and used to eliminate false positive samples/patients following initial screening of samples. In conclusion, we validated a specific method to investigate immunogenicity of Drug-X in human serum, performed in compliance with OECD-GLP and the latest developments in the immunogenicity field.

References

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