Obtaining a Sensitive MESO Scale Diagnostics (MSD®) Bridging Electrochemiluminescent Immunoassay for the Screening, Confirmation and Titration of Anti-Drug Antibodies in Human Serum using an Acid Dissociation Step

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OBJECTIVE

Validate a bridging anti-drug antibody (ADA) assay in human serum that uses an acid dissociation step while maintaining 100 ng/mL sensitivity. Implement an ADA assay with input from client to ensure mutually beneficial goals of validating an ADA method while attaining biologically relevant sensitivity with robust drug tolerance.

INTRODUCTION

Immunogenicity assay guidelines coupled with the importance of evaluation of patients' immune responses to therapeutic protein products create their own unique hurdles that need to be overcome. Here we present data outlining an Electrochemiluminescent bridging immunogenicity assay used to measure anti-drug antibodies in human serum using an acid dissociation step. The concern with using acid treatment in an immunogenicity assay is its potential deleterious effect on sensitivity and or drug tolerance. Here we will present assay performance parameters including: cut point determinations, sensitivity, drug tolerance, and selectivity demonstrating an assay that met guidance criteria and was useful to the client.

METHOD

This Electrochemiluminescent immunoassay detects anti-drug antibodies developed in patients after exposure to the drug and was developed with an acid dissociation pre-treatment step. The assay uses the bivalent binding capability of anti-drug antibodies to form a bridging complex with the biotinylated form and ruthenylated form of the drug generating RLU (relative light units) for the measurement of anti-drug antibodies in human serum. Samples and controls are diluted to their Minimum Required Dilution (MRD) of 1:5 before acetic acid is added to disassociate the drug from the ADA, followed by neutralization with an equal volume of Tris-Base. Neutralized samples are then incubated with the biotinylated and ruthenylated drug moieties to form the bridging immuno-complex. The complexes are then immobilized on a BSA-blocked streptavidin coated MSD plate and any unbound material is washed away. Finally MSD read buffer is added to the plate and a voltage applied to the plate by the MSD S 600 imager resulting in a detectable light signal directly proportional to the

METHODS (continued)

Table 1. Cut Point Determinations

FPER	Cut Point	Estimate Type	Cut Point Estimate
5.0%	Screening	Parametric 90% LCL	1.10
1.0%	Titration	Parametric	1.15
1.0%	Confirmatory (%INH by drug)	Parametric 80% LCL	7.0%

The parametric screening cut point factor of 1.10 is recommended to identify samples from normal subjects that are potentially positive for the presence of drug reactive ADA. Reactive samples may be classified as positive based on the %INH cut point of 7.0%. The titer for a confirmed positive sample can be determined using either the screening cut point factor of 1.10 (5.0% False Positive Error Rate (FPER)) or the higher titration cut point factor of 1.15 (1.0% FPER).

Table 2. Sensitivity Summary

Evaluation	Specification	Results
Sensitivity	≤100 ng/mL anti-Drug antibodies in human serum	≤ 6.00 ng/mL anti-Drug Antibodies

The sensitivity of the assay was determined and demonstrates a highly sensitive assay even with an acid disassociation step.

Table 3. Drug Tolerance Summary

Drug Tolerance Summary		
Evaluation	**Specification**	**Results**
Drug Tolerance	Highest tested drug concentration which failed to inhibit 100 ng/mL anti-Drug Ab below cut point.	2.00 µg/mL Drug
	Highest tested drug concentration which failed to inhibit 2500 ng/mL anti-Drug Ab below cut point	32.0 µg/mL Drug

Drug tolerance is important to demonstrate the assay is not negatively affected by the presence of drug while measuring the ADA. Drug tolerance of 2.00 µg/mL was attained when 100 ng/mL ADA was present (ratio of assay 20 Drug:1 ADA).

METHODS (continued)

Table 4. Selectivity Summary

Evaluation	Specification	Results
Selectivity - Normal Human Serum	Unsupplemented signal <100 ng/mL signal <2500 ng/mL signal	Met 100%
	Unsupplemented signal is below cut point	Met 80.0% in human serum samples. **ADB above cut point.**
	Supplemented samples (100 and 2500 ng/mL) are above screening cut point	Met 100% at both levels
	Surrogate antibody in individual matrices is within ±25.0% of assay buffer	Met 80% at 100 ng/mL and 90% at 2500 ng/mL anti-Drug antibody.

The parametric screening cut point factor of 1.10 is recommended to identify samples from normal subjects that are potentially positive for the presence of drug reactive ADA. Reactive samples may be classified as positive based on the %INH cut point of 7.0%. The titer for a confirmed positive sample.

SUMMARY AND CONCLUSION

The data presented show that a bridging anti-drug antibody immunogenicity assay that has an acid disassociation step can still maintain drug tolerance to decrease the chance of false negatives and sensitivity of ≤ 6.00 ng/mL anti-Drug Antibodies. In discussions with client we deemed this was sufficiently sensitive based on the time course in their protocol.

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