Immunogenicity Testing for Antibodies Directed Against Therapeutic Human Monoclonal Antibodies Using Electrochemiluminescent Detection

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ABSTRACT

Purpose:

Monitoring the immune response against human therapeutic monoclonal antibodies is an important component of pre-clinical and clinical trials in order to assess drug exposure, efficacy and safety. Detection of antibodies (analyte) directed against therapeutic antibodies (drug) is difficult due to the similarity of structure between drug and analyte and the high concentrations of drug present in serum. The purpose of this study was to develop a robust assay format that can be applied across species and drug compounds for rapid method development and validation.

Methods:

Each drug was conjugated with a ruthenium complex that emits light upon application of an electric potential (electro-chemiluminescence, ECL). A separate sample of the drug was also conjugated with biotin. Labeling ratios for both reagents were optimized to retain imunochemical reactivity. A rabbit polyclonal, affinity purified, antibody specific for each drug was used as a surrogate analyte. Rat, mouse, monkey or human sera containing the analyte were incubated with equimolar concentrations of biotin and ruthenium conjugated drug. The mixture was added to streptavidin coated plates equipped with electrodes to capture biotin-drug-analyte-ruthenium-drug complexes. ECL signals were measured on a Meso Scale Discovery Sector PR analyzer and normalized against a negative control run in every assay. Some assays required acid-dissociation to disrupt immune complexes prior to analysis. Reference interval, precision, limit of detection, tolerance to excess soluble drug, freeze-thaw and bench-top stability, and specificity of each assay was determined.

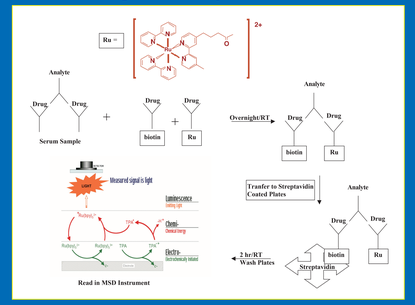
Conclusions:

An ECL bridging assay for detection of antibodies directed against human therapeutic antibodies was developed and validated in sera of multiple species. The high sensitivity and reproducibility of this technique allowed for low limits of detection (5 ng/mL), good precision (< 15%), wide dynamic range (10 ng/mL to 10 mcg/mL) and excellent tolerance to excess drug present in serum (drug to analyte ratio of 50). These versatile and straightforward assays can be effectively utilized to detect antibodies against therapeutic antibody drugs from pre-clinical development through clinical trails and post-marketing immunogenicity surveillance.

OBJECTIVES

1. Develop and validate a bridging electrochemiluminescent assay format to detect antibodies (analyte) against a therapeutic human monoclonal antibody (drug).
2. Maximize tolerance to high concentrations of drug present in serum samples.
3. Apply method across various drug candidates in various matrices.

Figure 1: Assay Schematic



Assay Optimization:

1. Maximize biotin-drug concentration (Limiting Factor: Streptavidin plate capacity).
2. Maximize ruthenium-drug concentration (Limiting Factor: Needs to be equimolar to biotin-drug concentration).
3. Optimize biotin and ruthenium conjugation ratios (Limiting Factor: Minimize interference with epitope presentation on drug).
4. Maximize incubation time (Limiting Factor: Turn-around time requirements).
5. Utilize acid dissociation if better drug tolerance is required (Limiting Factor: Low pH will denature proteins).

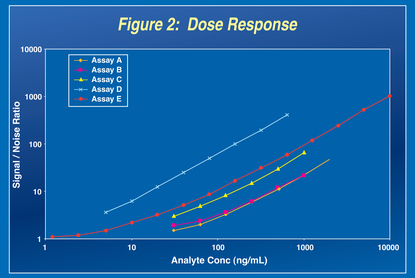
Table 1: Possible Combinations of Antibody and Drug

	Biotin- Drug (B)	Ruthenium- Drug (R)	Endogenous Drug (E)
Biotin-Drug (B)	B-Ab-B	B-Ab-R	B-Ab-E
Ruthenium-Drug (R)	R-Ab-B	R-Ab-R	R-Ab-E
Endogenous Drug (E)	E-Ab-B	E-Ab-R	E-Ab-E

Optimized Assay Methdology:

1. Specific anti-drug control antibodies for each assay were generated in rabbits and purified by affinity and negative selection chromatography.
2. Negative control was a pooled sample of the serum type being used.
3. Sample diluted to 10% into either 1% BSA buffer or 300 mM Acetic Acid.
4. Add 25 µL of 10% serum sample to 25 µL of 1 µg /mL of biotinylated drug and 25 µL of 1 µg/mL ruthenylated drug.
5. Incubate overnight at room temperature.
6. Transfer 50 µL to plates coated with streptavidin and incubate for 2 hr at room temperature with shaking.
7. Wash plate, add read buffer and analyze on MSD Sector PR instrument.
8. Convert average sample response (Electrochemiluminescence Units) to Signal / Noise ratio (Sample ECL / Neg Ctrl ECL).

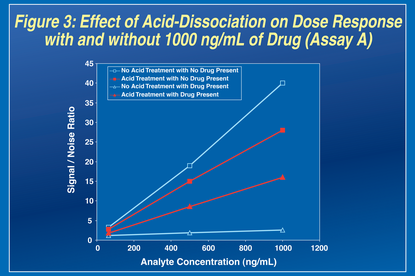
Figure 2: Dose Response



Observations of Dose Response Curves

- Sensitivity varied by assay up to 10 fold, probably due to different affinities of the control antibodies.
- Hook effect did not occur up to concentrations of 10,000 ng/mL of analyte.
- Response was linear between 10 ng/mL and 10,000 ng/mL of analyte.

Figure 3: Effect of Acid-Dissociation on Dose Response with and without 1000 ng/mL of Drug (Assay A)



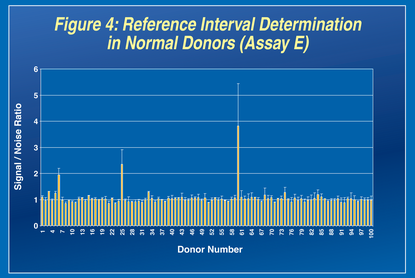
Methods for Acid Dissociation Pretreatment:

- Analyte diluted in serum.
- Drug added to serum at 0 and 1000 ng/mL.
- Serum pretreated with and without acid dissociation for 1 hour (pH 3.3).
- Samples brought to neutral pH and assayed.

Observations for Acid Dissociation Pretreatment (Assay A):

- Drug suppressed signal significantly.
- Acid pretreatment reduced overall signal when drug was not present.
- Acid pretreatment enhanced signal when interfering drug was present.

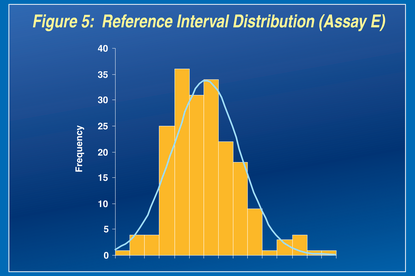
Figure 4: Reference Interval Determination in Normal Donors (Assay E)



Methods for Reference Interval Determination:

- Normal donor samples analyzed in multiple assays.
- Frequency distribution of aggregate data analyzed for normality.
- Data transformed by log or square root transformation if necessary to make more gaussian.
- One-sided reference interval determined from transformed data and reverted to S/N.

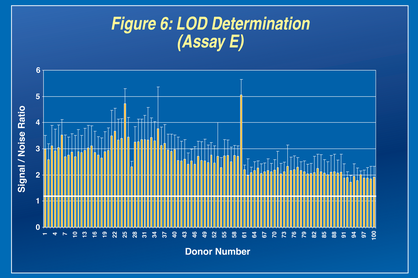
Figure 5: Reference Interval Distribution (Assay E)



Observations for Reference Interval Determination (Assay E):

- One hundred normal human sera demonstrated S/N ratios between 0.86 and 3.82.
- Three samples excluded as outliers.
- Distribution was gaussian.
- The upper limit of a 95% one-sided reference interval was 1.21 (mean + 1.645 SD).

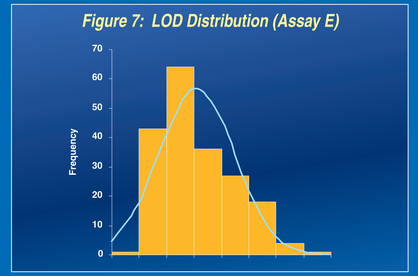
Figure 6: LOD Determination (Assay E)



Methods for LOD Determination:

- Normal donor samples spiked with LOD concentration and analyzed in multiple assays.
- All samples required to demonstrate S/N above reference interval (Threshold).
- Frequency distribution of aggregate data analyzed for normality.
- Data transformed by log or square root transformation if necessary to make more gaussian.
- Lower limit of responses determined from transformed data and reverted to S/N.

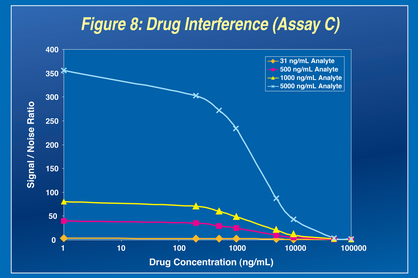
Figure 7: LOD Distribution (Assay E)



Observations of LOD Determination (Assay E):

- One hundred normal human serum spiked with 15 ng/mL of analyte demonstrated S/N ratios between 1.73 and 5.06.
- The lower limit of detection in the absence of drug was 15 ng/mL.
- Three samples were excluded as outliers.
- The distribution was gaussian.
- The minimum expected response at the LOD was 1.47 (mean – 2 SD).

Figure 8: Drug Interference (Assay C)



Methods for Drug Interference Determination:

- Analyte diluted to various concentrations in serum.
- Drug diluted to various concentrations in serum.
- Analyte and Drug dilutions mixed in 1:1 ratio.
- Concentrations shown are final neat serum concentrations.

Observations for Drug Interference Determination (Assay C):

- 500 ng/mL of analyte was detected in the presence of 100,000 ng/mL of drug.
- 31 ng/mL of analyte (LOD) was detected in the presence of 10,000 ng/mL of drug.

Table 2: Assay Characteristics

	Species	LOD (ng/mL)	Inter-Assay Precision at LOD (CV)	Acid Pre-treatment	Drug Tolerated (ng/mL) at LOD	Drug Tolerated (ng/mL) at 500 ng/mL of Analyte
Assay A	Mouse	63	8%	Yes	5,000	20,000
Assay B	Monkey	63	5%	Yes	1,000	5,000
Assay C	Human	31	14%	Yes	10,000	> 100,000
Assay D	Rat	5	4%	No	Not Determined	> 50,000
Assay E	Human	15	18%	No	> 5,000	> 50,000

CONCLUSIONS

- Antibodies against human monoclonal therapeutic antibodies were detected reliably with an electrochemiluminescent bridging assay.
- Good precision and rapid assay development was achievable in sera from different animal species.
- Limits of detection ranged from 5 to 63 ng/mL of rabbit anti-drug specific control antibodies.
- Greater than 50,000 ng/mL of drug was tolerated at antibody concentrations of 500 ng/mL in 3 out of 5 assays.
- Acid pretreatment of samples dissociated antibody-drug complexes and enhanced detection of antibodies.