

## Purpose.

Excess drug in serum samples can act to mask the detection of anti-drug antibodies (ADAs) in immunogenicity (IM) analyses. The drug-tolerance limit of an assay is interpreted as the highest drug concentration at which a sample containing a significant level of ADAs can be identified as positive. If the amount of drug in a sample is greater than the drug-tolerance limit of the assay, false-negative results may be reported during sample screening. Therefore, drug tolerance is a critical parameter to consider during method development. Bridging electrochemiluminescent (ECL) assays have become a popular IM platform. In the standard format, drug in samples may compete with both the capture and detection reagents. To decrease the potential of false-negative results, we sought to improve the drug-tolerance limit of a previously validated assay.

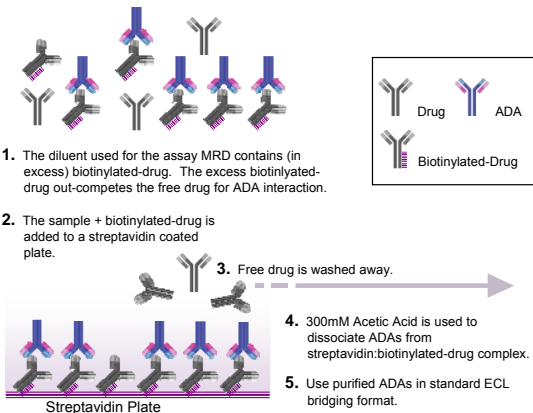
## Methods.

A sample incubation in the presence of excess immobilized-drug has been added to the standard bridging ECL method. The immobilized-drug acts to out-compete free drug in the sample while capturing ADAs.

Following a wash step to remove the drug present in the matrix, the ADA:immobilized-drug complex is disrupted using acid dissociation. The extracted ADAs are analyzed using the standard ECL IM format. Assay parameters were optimized in conjunction with the new method.

## Results.

A two-day assay has been developed to increase the drug tolerance of a bridging ECL format. The drug tolerance was raised from 5.00 µg/mL using the standard format to > 25.0 µg/mL at the low Positive Control (PC) level (100 ng/mL) and 100 µg/mL at the mid PC level (250 ng/mL). Selectivity in individual human serum samples was confirmed, and the sensitivity of the previously validated assay was maintained.



## Previously Validated Parameters

Relative Assay Sensitivity	63.0 ng/mL
Drug Tolerance	
Low PC (107 ng/mL)	5.00 ug/mL
High PC (13333 ng/mL)	10.0 ug/mL

## Positive Control Intra Assay Precision

	PC1 0.100 µg/mL Response	PC2 0.500 µg/mL Response	PC3 2.00 µg/mL Response
Rep 1	516	1869	9688
Rep 2	575	1802	7043
Rep 3	565	1976	6769
Rep 4	605	2082	7262
Rep 5	668	2174	9243
Rep 6	533	2341	9767
Mean	577	2040	8295
St Dev	54.4	200	1412
%CV	9.43	9.80	17.0

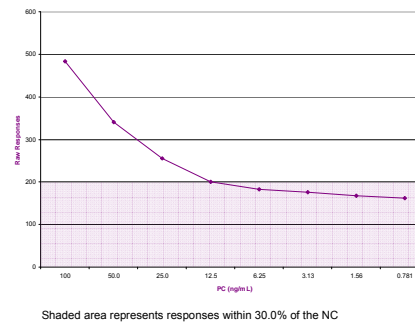
## Positive Control Inter-Assay Precision

	PC1 0.100 µg/mL Response	PC2 0.500 µg/mL Response	PC3 2.00 µg/mL Response
Assay12	377	1446	5540
	361	1457	5363
Assay14	603	2451	7168
	614	2657	6262
Assay15	629	2441	11215
	618	2411	9500
Assay17	676	1726	6639
	592	2202	6618
Assay19	454	1596	7396
	490	2004	7717
Assay20	523	2119	7109
	513	2045	6957
Assay22	599	2115	7468
	576	2199	7333
Assay23	440	1754	6525
	535	2001	5659
Mean	537	2039	7154
St Dev	92.6	364	1468
%CV	17.2	17.8	20.5

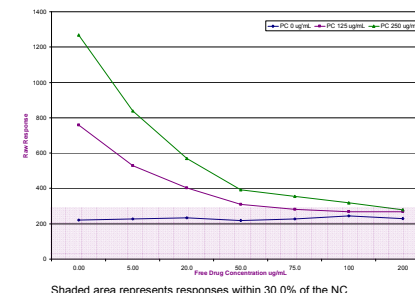
## Selectivity in Normal Human Serum

	Raw Response	%Diff from PC1
NC	0.00 µg/mL	246
PC1	0.100 µg/mL	720
PC2	0.500 µg/mL	2657
PC3	2.00 µg/mL	10431
BLANK 1	218	
PC1 SPIKE 1	669	-7.05
BLANK 2	279	
PC1 SPIKE 2	765	6.22
BLANK 3	203	
PC1 SPIKE 3	714	-0.868
BLANK 4	198	
PC1 SPIKE 4	668	-7.26
BLANK 5	224	
PC1 SPIKE 5	644	-10.6
BLANK 6	215	
PC1 SPIKE 6	747	3.72
BLANK 7	233	
PC1 SPIKE 7	674	-6.43
BLANK 8	225	
PC1 SPIKE 8	717	-0.452
BLANK 9	211	
PC1 SPIKE 9	636	-11.7
BLANK 10	225	
PC1 SPIKE 10	705	-2.05

## Relative Assay Sensitivity



## Drug Tolerance



Shaded area represents responses within 30.0% of the NC

PC µg/mL	Drug µg/mL	Mean Raw Response	Result
0.00	0.00	222	negative
0.00	5.00	228	negative
0.00	20.0	235	negative
0.00	50.0	219	negative
0.00	75.0	227	negative
0.00	100	244	negative
0.00	200	229	negative
0.125	0.00	758	potentially positive
0.125	5.00	528	potentially positive
0.125	20.0	403	potentially positive
0.125	50.0	309	potentially positive
0.125	75.0	281	negative
0.125	100	268	negative
0.125	200	268	negative
0.250	0.00	1268	potentially positive
0.250	5.00	838	potentially positive
0.250	20.0	570	potentially positive
0.250	50.0	392	potentially positive
0.250	75.0	356	potentially positive
0.250	100	318	potentially positive
0.250	200	279	negative

Samples with responses > 30% of NC mean are designated as *potentially positive*.

## Conclusion.

We have utilized an effective way to increase drug tolerance in ECL IM assays. This will allow more sensitive detection of ADAs in presence of high levels of the drug during sample screening. This method may minimize the need to wait the determined washout period for analysis of ADA in study samples and decrease the rate of false-negative results during screening.