Development of an Enhanced Sensitivity Immunogenicity (ADA) Assay on the Next Generation SMC™ Technology

Anitaben Tailor[1], Robert Hardcastle[2], Daniel Garcia-West[2], Nicholas White[3], Jo Goodman[3]
[1]MilliporeSigma, 400 Summit Drive, Burlington, 01803 MA, [2]Merck, Frankfurter Straβe 250 Darmstadt, 64293, Germany, [3]AstraZeneca, Granta Park, Cambridge, CB21 6GH UK

Introduction

Drug immunogenicity and the detection of anti-drug antibodies (ADA) have an important role in the drug discovery process for potential new therapeutics. The clinical effects of these immune responses can affect pharmacokinetics, pharmacodynamics, safety, or efficacy. Detection and analysis of ADA formation is crucial for any therapeutic protein product development program.

Consequently, regulatory agencies are looking to understand the implications of immunogenicity and are directing the industry to integrate programs for immunogenicity risk management starting in early phase drug development in clinical and pre-clinical. Agencies are stipulating that screening and confirmatory IgG and IgM ADA assays should achieve a sensitivity of at least 100 nanograms per millilitre (ng/mL). Assays developed to assess IgE ADA should have sensitivity in the high picograms per millilitre (pg/mL) to low ng/mL range.

Merck's propriety Single Molecule Counting (SMC™) immunoassay technology SMC™ technology can support all phases of immunogenicity testing using digital counting on the SMCxPRO™ high-sensitivity instrument for low-level protein detection.

SMC™ advantages include, ultrasensitivity down to pg/mL detection for low-affinity ADA and reduced need for dilutions as well as a wide dynamic range for detection of high-affinity ADA with minimal matrix interference. All ADA subtypes can be detected including IgM and IgE and tolerance to high drug concentrations in sample is well tolerated. Reduced wash steps for detection of low-affinity antibodies offers advantages and helps reduce assay time.

The SMC™ Immunogenicity Assay Development Kit (Cat. No. 03-0175-00) and the SMCxPRO system was used to assess the possibility for ultra-sensitive detection of ADA from serum samples in clinical samples.

Figure 1: SMCxPRO™
Utility of the high sensitivity fluorescent based platform, to measure high and low affinity ADA levels in samples.

Methods

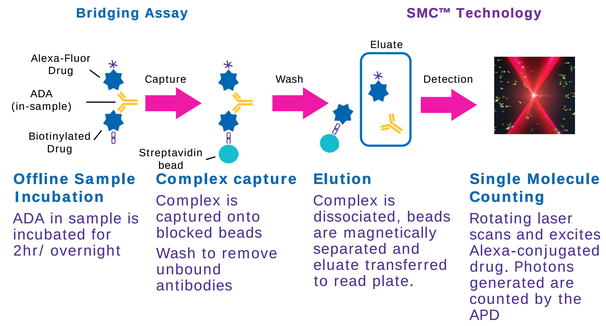

Figure 2: SMC™ Bridging Immunoassay Workflow
This figure illustrates the typical immunogenicity ADA bead based assay work flow. A bridging immunoassay complex is captured onto beads. The complex is disassociated from the bead and the eluate is read on the SMCxPRO™.

Results (I)

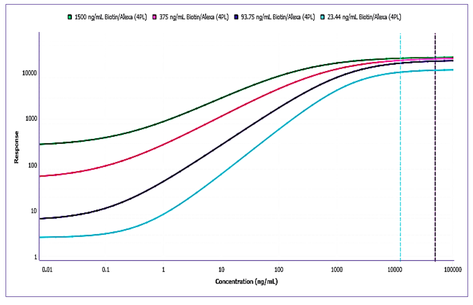

Figure 3: Titration of Biotin-Drug and Alexa Fluor Drug
This figure illustrates the equimolar titration of biotinylated Drug and Alexa-Fluor Drug against a positive Rabbit PAb (ADA). The graphs represent 12-point ADA curves at each titration, with < 20% CV

ADA ng/mL	1500 ng/mL B + F	375 ng/mL B + F	62.75 ng/mL B + F	23.44 ng/mL B + F
0	1	1	1	1
0.05	1	2	1	1
0.19	2	2	1	1
0.76	3	3	2	2
3.05	6	9	7	8
12.00	10	25	28	29
49	19	44	96	142
195	41	93	200	380
781	64	181	431	1070
3,125	71	295	923	2151
12,500	70	309	1282	3685

Table 1: Signal: Noise Ratio The table illustrates S:N ratio at each of the Biotinylated Drug and Alexa Fluor drug titration. 23.44 ng/mL demonstrated the optimal S:N ratio and dynamic range.

Results (II)

A

ADA ng/mL	20% Matri	10% Matri	5% Matrix	0% Matri
0.00	68	39	22	2
0.05	72	37	20	80
0.15	68	35	22	162
0.46	68	47	23	357
1.37	92	49	25	988
4.12	110	60	34	2090
12.35	206	112	55	2946
37.04	491	230	125	5892
111.11	897	647	265	8293
333.33	2318	1767	999	8066
1000.00	3363	2371	1875	8235
3000.00	4330	3791	2547	6894

B

ADA ng/mL	20% Matrix	10% Matrix	5% Matrix	0% Matrix
0.00	1	1	1	1
0.05	1	1	1	33
0.15	1	1	1	67
0.46	1	1	1	147
1.37	1	1	1	407
4.12	2	2	2	861
12.35	3	3	3	1214
37.04	7	6	6	2428
111.11	13	17	12	3417
333.33	34	45	45	3323
1000.00	50	61	85	3393
3000.00	64	97	116	2840

Table 2: Matrix Tolerance Assay was run in 20%, 10%, 5% and 0% matrix to determine MRD **A)** represents Response Events at each % matrix and **B)** represents Signal-to-Noise ratio

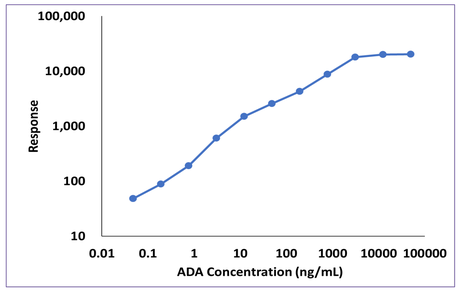

Figure 4: Matrix Tolerance
MRD (1 in 5 [20% Matrix]) 0.025 µg/mL Biotin-Drug: 0.025 µg/mL Alexa-Drug was considered optimal

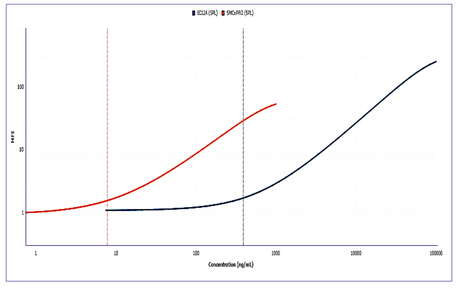

Figure 5: SMC Vs. ECLIA Comparison
Sensitivity improved 10-fold over the traditional ECLIA method from 195 ng/mL to 20 ng/mL.

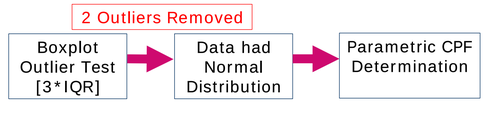

Figure 6: Cut Point Assessment
Cut point was determined by screening 50 drug naïve cynomolgus samples.
- SCPF 2.98 (1% FPR*)
  * More stringent SCPF for non-clinical

Results (III)

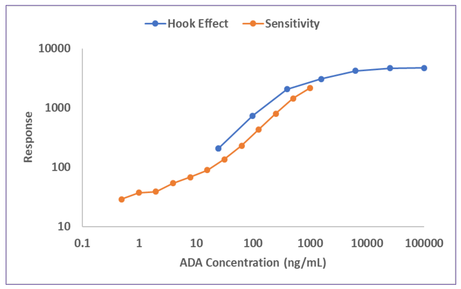

Figure 7: Hook Effect & Sensitivity
Current ADA assay demonstrated no evidence of hook effect up to 100,000 ng/mL with low sensitivity to pg/mL level

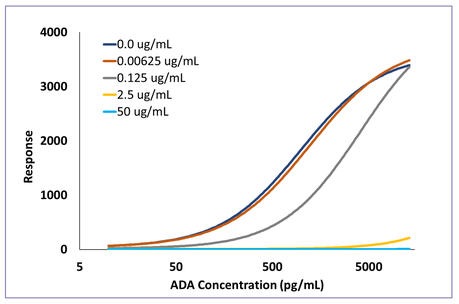

Figure 8: Drug Tolerance
Assay demonstrated reduced tolerance to circulating therapeutic. Assay could detect 97.7 ng/mL ADA in the presence of 0.125 µg/mL MAb

Summary

- The SMC™ technology offers 10-fold improvement in sensitivity over current gold standard assay.
- The assay demonstrated reduced matrix tolerance and demonstrated equivalent drug tolerance to current assay.
- Relative to other assay platforms, the assay utilized reduced reagent consumption whilst maintaining the dynamic range of the assay.
- The current assay was an unoptimized assay, lending itself to improvement following optimization

Conclusion

The improved sensitivity may lead to early detection of primary ADA response prior to class type switching and affinity maturation

References:
1. FDA guidance - Immunogenicity Testing of Therapeutic Protein Products, Developing & Validating Assays for Anti-Drug Antibody Detection. Jan 2019