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

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## 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.

MilliporeSigma'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

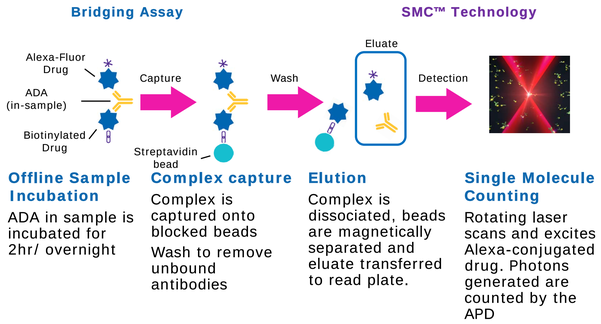


**Figure 2: SMC™ Bridging Immunoassay Workflow**
This figure illustrates the typical immunogenicity ADA bead based assay work flow. A bridging immunoassay complex is captured onto beads. The complex is disassociated from the bead and the eluate is read on the SMCxPRO™.

## Results (I)

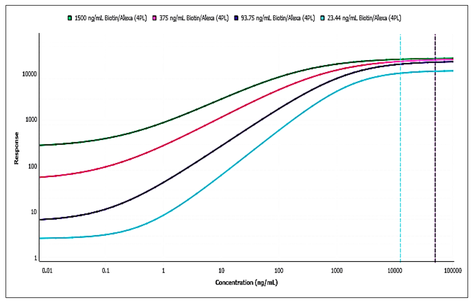


**Figure 3: Titration of Biotin-Drug and Alexa Fluor Drug**
This figure illustrates the equimolar titration of biotinylated Drug and Alexa-Fluor Drug against a positive Rabbit PAb (ADA). The graphs represent 12-point ADA curves at each titration, with < 20% CV

| 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

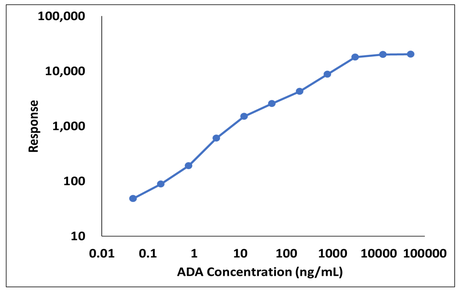


**Figure 4: Matrix Tolerance**
MRD (1 in 5 [20% Matrix]) 0.025 µg/mL Biotin-Drug: 0.025 µg/mL Alexa-Drug was considered optimal

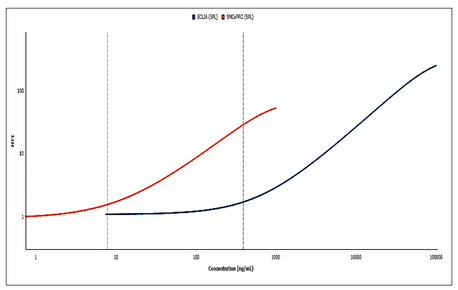


**Figure 5: SMC Vs. ECLIA Comparison**
Sensitivity improved 10-fold over the traditional ECLIA method from 195 ng/mL to 20 ng/mL.

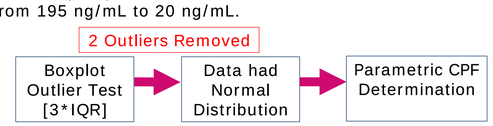


**Figure 6: Cut Point Assessment**
Cut point was determined by screening 50 drug naïve cynomolgus samples.
- SCPF 2.98 (1% FPR*)
  * More stringent SCPF for non-clinical

## Results (III)

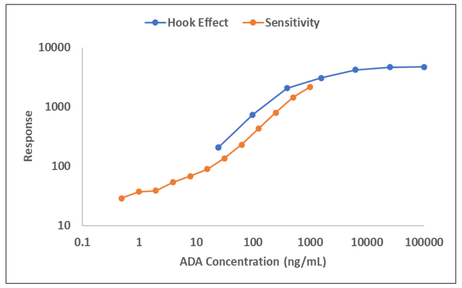


**Figure 7: Hook Effect & Sensitivity**
Current ADA assay demonstrated no evidence of hook effect up to 100,000 ng/mL with low sensitivity to pg/mL level

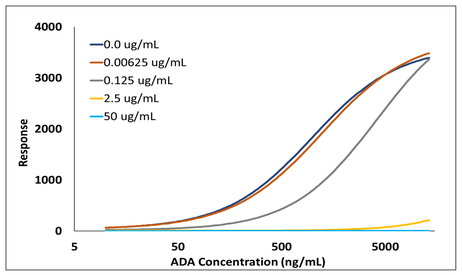


**Figure 8: Drug Tolerance**
Assay demonstrated reduced tolerance to circulating therapeutic. Assay could detect 97.7 ng/mL ADA in the presence of 0.125 µg/mL MAb

## 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