



# Increase Drug Tolerance of Immunogenicity Assay by Affinity Purification with a Novel Technical Platform

## Authors

**Xiguang Li, Edward Brewer, KC Van Horne**

*Tandem Labs, a LabCorp company*

## Purpose

Preclinical and Clinical safety studies require dosing subjects with high concentrations of therapeutic proteins while facing the challenge of detecting low level antidrug antibodies (ADA) in patients with high residual drug concentrations<sup>[1-3]</sup>.

This poster provides a method for detecting low level ADAs in the presence of high drug concentrations.

## Methods

### 1. KEY MATERIALS:

- CNBr-activated Sepharose™ 4B (GE Healthcare)
- High Capacity Streptavidin Agarose Resin (Thermo Scientific)
- Drug (normal human IgG, Sigma-Aldrich®)
- Analyte (Goat anti human IgG, Thermo Scientific)
- MultiScreen<sub>HTS</sub> Vacuum Manifold and MultiScreen-Mesh 96-well filter plates (EMD Millipore)



## Methods (continued)

### 2. OPERATION STEPS:

#### **Day 1: Sample Pretreatment**

Step 1: Serum sample mix with CNBr-drug or Streptavidin-biotinylated-drug resin/PBS (1:1 volume) overnight.

#### **Day 2: Sample Analysis**

Step 2: Transfer overnight mixing samples to a 96 well filter plate.

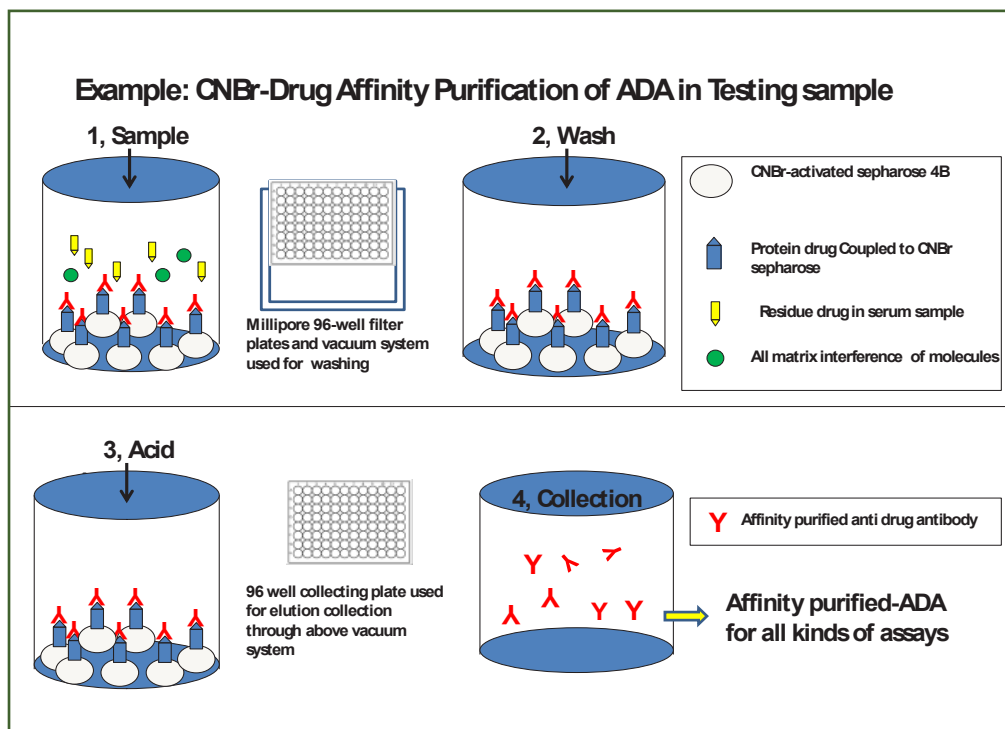
Step 3: Wash filter plate 3 times through Millipore vacuum system.

Step 4: Acid dissociate the ADA from drug-resin within filter plate for 10 minutes.

Step 5: Collect the dissociating ADA to a 96 well neutralization plate through the Millipore vacuum system.

Step 6: Add samples to MSD; ELISA or cell plates, continue assays with standard protocols.

#### **EXAMPLE: CNBr-Drug Affinity Purification of ADA Testing sample**



**Affinity Purified ADA Detected by MSD Regular Bridging Platform:**  
coating MSD plate with biotinylated drug and detected by sTag-drug.



## Methods (continued)

**TABLE 1:**  
**No Resin pre-treatment Sample, drug tolerance at about 10µg/mL.**

No Resin	NC	PC 0.5ug/mL	PC-0.5ug/mL drug 500 ug/mL	PC-0.5ug/mL drug 100ug/mL	PC-0.5ug/mL drug 10ug/mL	PC-0.5ug/mL drug 1up/mL
Mean	218	43335	106	137	281	768
%CV	9.5	1.6	4.7	0.5	1.0	1.8
PC/NC	1.00	199	0.48	0.63	1.29	3.52

**TABLE 2:**  
**Streptavidin-Biotin-drug Resin pre-treated sample, drug tolerance is ~ 500µg/mL.**

Streptavidin- Biotin-hlgG Resin	NC	PC 0.5ug/mL	PC-0.5ug/mL drug 500 ug/mL	PC-0.5ug/mL drug 100ug/mL	PC-0.5ug/mL drug 10ug/mL	PC-0.5ug/mL drug 1up/mL
Mean	145	38069	335	1195	5268	13053
%CV	7.8	0.1	0.6	0.9	1.1	0.0
PC/NC	1.00	263	2.31	8.27	36.45	90.33

**TABLE 3:**  
**CNBr-drug Resin pre-treatment sample, drug tolerance is >500µg/mL.**

CNBr-hlgG Resin	NC	PC 0.5ug/mL	PC-0.5ug/mL drug 500 ug/mL	PC-0.5ug/mL drug 100ug/mL	PC-0.5ug/mL drug 10ug/mL	PC-0.5ug/mL drug 1up/mL
Mean	284	13057	1562	4703	8856	106287
%CV	5.2	0.8	1.5	1.3	1.4	0.4
PC/NC	1.00	46	5.50	16.57	31.21	37.66



## Results

---

### **Drug Tolerance for a Positive Control (anti hIgG antibody) at 0.5 µg/mL in matrix:**

**Table 1,** No resin pre-treated samples:

The drug tolerance is around 10µg/mL with a PC/NC ratio of 1.29.

**Table 2,** Streptavidin-Biotinylated drug (hIgG) resin pre-treated samples:

The drug tolerance is around 500µg/mL with a PC/NC ratio of 2.31.

**Table 3,** CNBr-drug (hIgG) resin pre-treated samples:

The drug tolerance is around 500µg/mL with a PC/NC ratio of 5.50.

## Conclusions

---

Drug coupling to commercial available resins, Streptavidin Agarose and CNBr-activated sepharose™4B, with a 96well filter plate washing system has generated a homemade drug specific affinity purification chromatography system.

Improvements our method provides in testing for ADAs include:

1. Uses a matrix-free assay, rather than a traditional matrix-based assay, removing some of the matrix and residual drug effects.
2. Uses a 96-well filter plate, rather than traditional chromatography, increasing throughput and saving time.
3. Demonstrated increasing drug tolerance from 10 to 500µg/mL for a Positive Control ADA at 0.5µg/mL.

## References

---

- [1] Mire-Sluis AR, Barrett YC, Devanarayan V, Koren E, Liu H, Maia M, Parish T, Scott G, Shankar G, Shores E, Swanson SJ, Taniguchi G, Wierda D, Zuckerman LA. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. *J Immunol Methods*. 2004 Jun;289(1-2):1-16. PubMed PMID: 15251407.
- [2] Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher R, Finco-Kent D, Fiscella M, Gorovits B, Kirschner S, Moxness M, Parish T, Quarmby V, Smith H, Smith W, Zuckerman LA, Koren E. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. *J Pharm Biomed Anal*. 2008 Dec 15;48(5):1267-81. Epub 2008 Sep 19. Review. PubMed PMID: 18993008.
- [3] Gupta S, Devanarayan V, Finco D, Gunn GR 3rd, Kirshner S, Richards S, Rup B, Song A, Subramanyam M. Recommendations for the validation of cell-based assays used for the detection of neutralizing antibody immune responses elicited against biological therapeutics. *J Pharm Biomed Anal*. 2011 Jul 15;55(5):878-88. Epub 2011 Apr 6. Review. PubMed PMID: 21531522