# Development of PK and Anti-Drug Antibody Assay with High Drug Tolerance for Bevacizumab (Avastin®) and its Biosimilars

S. Kar, N. Chavan, C. Sheldon, R. Islam
Celerion, Lincoln, NE USA

celerion
Translating Science to Medicine

## Introduction

Bevacizumab (Avastin®) is a recombinant humanized monoclonal antibody approved globally for treatment of multiple types of cancer. It is a popular target for biosimilar developers as the patents on Avastin® will expire in July 2019 in US and in January 2022 in Europe.

Anti-Drug Antibody (ADA) immunogenicity and pharmacokinetic (PK) assays for Bevacizumab are challenging due to the fact that a high level of drug is expected in clinical samples. The mean trough concentration range is 127 ± 29 µg/ml (range 77–155), and the mean peak concentration is 149 ± 13 µg/ml (range 113–157). In addition, endogenous VEGF can interfere with the detection of Bevacizumab and ADA. This high level of drug concentration combined with VEGF interference necessitates the development of de novo, highly reliable assays for the biosimilar.

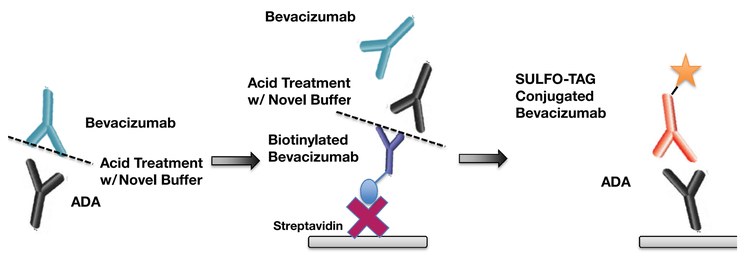
Here, we present an ELISA PK assay and an immunogenicity assay that utilizes solid phase extraction with double acid dissociation (SPEAD) on the Meso Scale Discovery (MSD) platform.

## Methods

### PK Assay Format

The PK assay for total bevacizumab is a sandwich ELISA, wherein $VEGF_{165}$ is the capture ligand and anti-bevacizumab isthe detection antibody with HRP detection. A proprietary buffer was used to remove VEGF from study samples to minimize VEGF interference.

**Figure 1. ADA Assay Format**




### ADA Assay Format

The Immunogenicity method utilized a modified solid phase extraction with acid dissociation (SPEAD) assay with Sulfo-Tag detection. Samples containing bevacizumab and ADA were pre-dissociated and neutralized using proprietary buffers before incubation with biotinylated bevacizumab on a streptavidin coated plate. The ADA was then dissociated, neutralized, and coated onto a MSD plate and detected using Sulfo-Tag labeled bevacizumab.

## Results

**Table 1. Precision and Accuracy of Bevacizumab PK Assay**

| Date | Batch | LLOQ (50 ng/mL) | QC A (150 ng/mL) | QC B (800 ng/mL) | QC C (1500 ng/mL) | ULOQ (2000 ng/mL) |
|---|---|---|---|---|---|---|
| 11-Apr-2018 | 8 | 57.4 | 164 | 904 | 1580 | 1990 |
| | | 50.4 | 168 | 799 | 1500 | 1930 |
| | | 54.5 | 141 | 786 | 1420 | *No Value |
| 11-Apr-2018 | 9 | 53.4 | 151 | 812 | 1540 | 2190 |
| | | 49.6 | 164 | 802 | 1580 | 2270 |
| | | 49.5 | *No Value | 772 | 1660 | 2240 |
| 13-Apr-2018 | 10 | 51.7 | 159 | 795 | 1430 | 1950 |
| | | 53.2 | 149 | 778 | 1440 | 1650 |
| | | 44.8 | 131 | 755 | 1320 | 1690 |
| 13-Apr-2018 | 11 | 50.6 | 150 | 822 | 1620 | 1990 |
| | | 51.3 | 149 | 823 | 1410 | 1920 |
| | | 45.3 | 127 | 797 | 1440 | 1720 |
| 13-Apr-2018 | 12 | 58.5 | 162 | 821 | 1580 | 2060 |
| | | 64.0 | 167 | 833 | 1480 | 1750 |
| | | 51.1 | 146 | 764 | 1430 | 1620 |
| Mean | | 52.4 | 152 | 804 | 1500 | 1930 |
| S.D. | | 4.91 | 12.9 | 35.9 | 94.4 | 217 |
| %CV | | 9.4 | 8.5 | 4.5 | 6.3 | 11.2 |
| %Theoretical | | 104.8 | 101.3 | 100.5 | 100.0 | 96.5 |
| %Bias | | 4.8 | 1.3 | 0.5 | 0.0 | -3.5 |
| n | | 15 | 14 | 15 | 15 | 14 |
| | | | | | | |
| Overall %CV | | 8.0 | | | | |

* QMV=questionable multiple values

**Table 2. Dilution Linearity of Bevacizumab**

| | DF=20 QC D 20000 ng/mL | DF=200 QC E 250000 ng/mL |
|---|---|---|
| | 20200 | 231000 |
| | 20300 | 243000 |
| | 19500 | 244000 |
| | 16400 | 267000 |
| | 17900 | 274000 |
| | 17400 | 199000 |
| | 18600 | 243000 |
| Mean | | |
| % CV | 8.7 | 11.1 |
| % | | |
| Theoretical | 93.0 | 97.2 |
| n | 6 | 6 |

**Table 3. Matrix Effect Testing of Bevacizumab PK Assay**

| | | Low Spike 50.0 ng/mL | | | High Spike 1600 ng/mL | | |
|---|---|---|---|---|---|---|---|
| | Mean Basal Level | Expected Concentration | Measured Concentration | | Expected Concentration | Measured Concentration | |
| Lot# | ng/mL | ng/mL | ng/mL | % Dev. | ng/mL | ng/mL | % Dev. |
| 1 | 4.81 | 54.8 | 50.2 | -8.4 | 1605 | 1610 | +0.3 |
| 2 | 22.8 | 72.8 | 66.1 | -9.2 | 1623 | 1590 | -2.0 |
| 3 | 60.7 | 111 | 100 | -9.7 | 1661 | 1660 | -0.0 |
| 4 | 0.0 | 50.0 | 42.9 | -14.2 | 1600 | 1720 | +7.5 |
| 5 | 24.8 | 74.8 | 78.0 | +4.3 | 1625 | 1630 | +0.3 |
| 6 | 46.6 | 96.6 | 88.6 | -8.3 | 1647 | 1610 | -2.2 |
| 7 | 0.0 | 50.0 | 39.8 | -20.4 | 1600 | 1510 | -5.6 |
| 8 | 62.5 | 113 | 109 | -3.1 | 1663 | 1660 | -0.2 |
| 9 | 0.0 | 50.0 | 48.8 | -2.4 | 1600 | 1600 | +0.0 |
| 10 | 65.5 | 116 | 123 | +6.5 | 1666 | 1510 | -9.3 |

**Table 4. Freeze-Thaw Stability (4 cycles) of Bevacizumab PK**

| | FT LLOQ | FT ULOQ |
|---|---|---|
| | 50.0 ng/mL | 2000 ng/mL |
| | 41.1 | 1870 |
| | 48.1 | 1970 |
| | 51.7 | 1760 |
| | 46.0 | 1860 |
| | 49.7 | 1710 |
| | 51.5 | 2150 |
| | | |
| Mean | 48.0 | 1890 |
| % CV | 8.4 | 8.4 |
| % Theoretical | 96.0 | 94.5 |
| n | 6 | 6 |

**Figure 2. Sensitivity of Bevacizumab ADA Assay (7.8 ng/mL)**

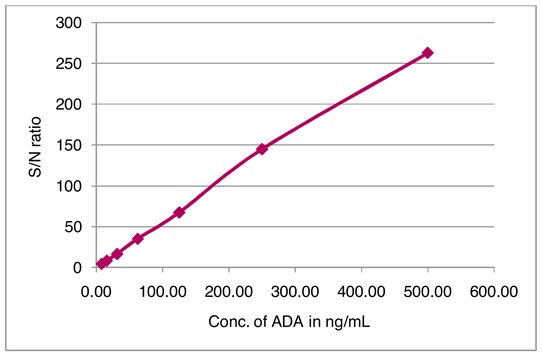
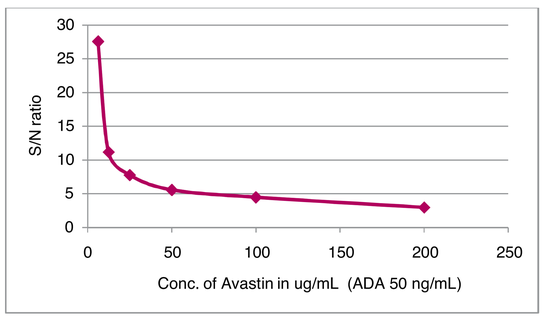



**Figure 3. Drug Tolerance of Bevacizumab ADA Assay (50 ng/mL ADA detectable with 100 µg/mL of Drug)**




| µg/mL Drug | S/N (50 ng/mL ADA) |
|---|---|
| 200 | 3 |
| 100 | 4.5 |
| 50 | 5.6 |
| 25 | 7.8 |
| 12.5 | 11.2 |
| 6.25 | 27.6 |

## Conclusion & Future Work

The results indicate the PK and ADA assays for Bevacizumab are “validatable” with sensitivity and drug tolerance that meets FDA requirements.

Future experiments will validate the assays according to FDA Bioanalytical Guidance for PK and immunogenicity assays for Bevacizumab and its biosimilars.

## Novel Aspect

Development of novel dissociation and neutralization buffers for the ADA assay allowed removal of high levels of tightly bound bevacizumab to improve drug tolerance. Combined with a double dissociation and electrochemiluminescence, we improved assay sensitivity over conventional immunogenicity methods.