# A Novel SPEAD-Based Approach to Overcome Steric Hindrances in GLP-1 Peptide Therapeutic ADA Assay

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POSTER # P05

## INTRODUCTION

Metabolic therapeutics, particularly glucagon-like peptide-1 (GLP-1)–based peptides, are experiencing rapid growth within the drug development landscape. ADA assays for peptide therapeutics present unique bioanalytical challenges. Owing to the small size of peptides and their limited epitope availability, the most common assay configuration involves capture of the peptide therapeutic followed by detection using species-specific secondary antibodies. While this format is technically feasible, it frequently introduces elevated nonspecific background, ultimately compromising assay sensitivity and robustness. This presentation describes a novel immunogenicity assay methodology that integrates solid-phase extraction with acid dissociation (SPEAD) and a protein A/G/L capture step. The protein A/G/L binds to the Fc portion of the antibodies, thereby positioning the ADA in the optimal orientation to allow binding to the ruthenium-labeled peptide, increasing assay sensitivity and specificity. This configuration enables direct detection using the peptide therapeutic itself, eliminating the need for a less-specific anti-species detection reagent and thereby reducing assay background.

## METHODS

**Labeling of the peptide with Biotin and Ruthenium:** The metabolic peptide therapeutic was successfully labeled with biotin and ruthenium with dialysis cassettes, using a challenge ratio of 20 and 8 for the biotin and ruthenium, respectively.

**Attempted Assay Formats:**

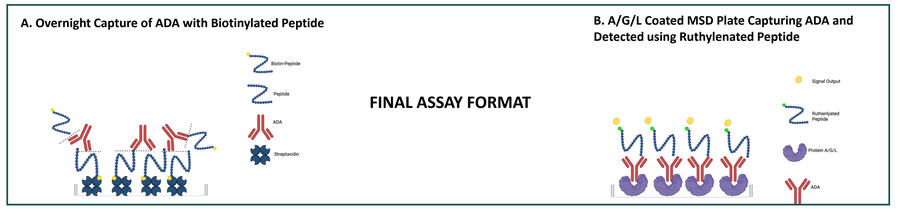
| Bridging MSD Assay-Incubation with B- and Ru- labeled Peptides in MasterMix | Step-Wise MSD Assay with B- and Ru- labeled Peptides incubated sequentially | Overnight Passive Peptide Coat with Ru- labeled peptide detection | Overnight Passive Peptide Coat with 2-step Detection, B- labeled peptide followed by Streptavidin-Sulfotag | Overnight Passive Peptide Coat of ELISA plate with 2-step Detection, B- labeled peptide followed by Streptavidin-HRP |
|---|---|---|---|---|

**Final Assay Procedure:**

**Day 1:** Streptavidin ELISA plate is coated with Biotinylated Peptide. Samples are prepared, diluted to MRD20 in Low Cross Buffer, and transferred to the ELISA Plate to incubate overnight at 2-8°C with shaking. A Standard MSD Plate is also coated with Protein A/G/L overnight at 2-8°C with shaking.

**Day 2:** The A/G/L coated MSD is washed and blocked with Blocker Casein. The sample capture streptavidin plate is washed, and ADA is eluted using acetic acid. The acidified samples are neutralized with tris and transferred to the A/G/L coated/blocked MSD plate. Last, the samples are detected using ruthenylated peptide and then read on an MSD Sector Imager.

## RESULTS



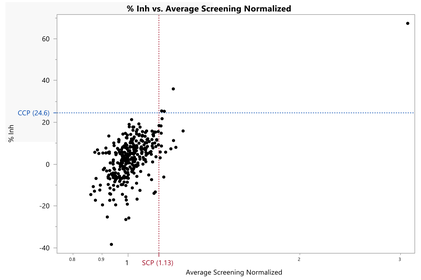




**Figure 1: Specificity Plot %Inhibition by Averaged Screening Normalized Signal**

**A cut point was determined by analyzing 54 treatment naïve individuals six times by two analysts.**
**Three technical outliers (%CV> 20) excluded from screening CP analysis. No analytical or biological outliers identified. Screening data was not normally distributed; 95th percentile point estimate used.**
**Six technical outliers (%CV>20) excluded from confirmatory CP analysis. No analytical or biological outliers identified. Confirmatory data was not normally distributed; 99th percentile point estimate used.**

**Figure 2: Screening Sensitivity**

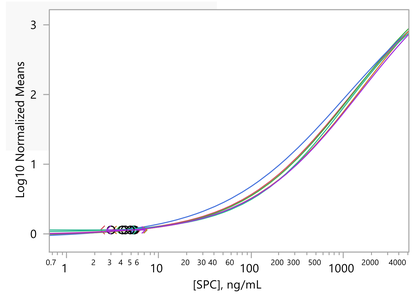



**Figure 3: Confirmatory Sensitivity**

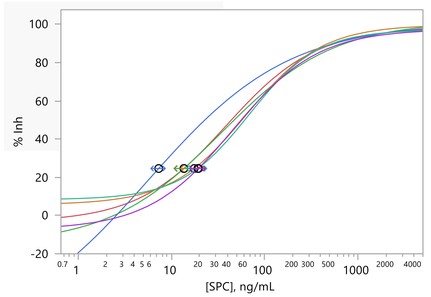



**Figure 2 and Figure 3: Sensitivity in the Screening and Confirmatory assay was evaluated in 6 runs by 2 analysts over 3 days. The sensitivity curves ranged from 5000ng/mL to 2.44ng/mL SPC in neat matrix prior to MRD dilution.**

## RESULTS

| Screening Cut Point | Confirmatory Cut Point | Titer Cut Point |
|---|---|---|
| 1.13 S/N | 24.6% INH | 1.25 S/N |
| False Positive Rate | False Positive Rate | False Positive Rate |
| 4.7% | 0.9% | Estimate 0.1% |

| | Tier | Sensitivity (ng/mL) | LPC Estimation (ng/mL) |
|---|---|---|---|
| Sensitivity of Six Curves | Screen | 4.5 | 5.8 |
| | Confirm | 15.1 | 21.5 |

| | SPC Concentration | Screen %CV | | Confirm %CV |
|---|---|---|---|---|
| | | LUM | S/N | %Inh |
| Control Inter-Assay Precision over 24 Cut Point and Sensitivity Plates | 5000ng/mL | 16.6 | 14.6 | 0.8 |
| | 100 ng/mL | 11.4 | 9.3 | 5.9 |
| | 0 ng/mL | 6.6 | N/A | 7.8 (LUM) |

| | Population | SPC Conc | Screening Results | Confirmatory Results |
|---|---|---|---|---|
| Selectivity | 10 treatment naïve individuals (5M/5F) | 5000 ng/mL | 10/10 (100%) Screen Pos | 10/10 (100%) Confirm Pos |
| | | 25 ng/mL | 10/10 (100%) Screen Pos | 10/10 (100%) Confirm Pos |
| | | 0 ng/mL | 10/10 (100%) Screen Neg | 10/10 (100%) Confirm Neg |
| | 5 Lipemic individual samples | 5000 ng/mL | 5/5 (100%) Screen Pos | 4/5 (80%) Confirm Pos |
| | | 25 ng/mL | 5/5 (100%) Screen Pos | 4/5 (80%) Confirm Pos |
| | | 0 ng/mL | 4/5 (80%) Screen Neg | 5/5 (100%) Confirm Neg |

## CONCLUSIONS

Metabolic peptide therapeutics present distinct bioanalytical challenges in the development of immunogenicity assays. Conjugation of detection labels such as biotin and ruthenium to these peptides is often problematic due to the limited availability of primary amines, and in some cases, labeling may not be feasible at all. Furthermore, the small molecular size and limited epitope availability of metabolic peptides preclude the use of traditional bridging ADA assay formats. The method described herein, together with preliminary analytical data, demonstrates that a solid-phase extraction with acid dissociation (SPEAD) approach combined with protein A/G/L direct capture enables a highly sensitive and reproducible assay, effectively addressing a critical gap in the bioanalytical assessment of metabolic peptide therapeutics