

Pre-clinical analysis of therapeutic antibodies of differing allotypes in rodent plasma using MSIA D.A.R.T.'s technology, an integral part of the universal ligand binding mass spectrometric immunoassay (LB-MSIA) workflow

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Key Words

Ligand Binding Mass Spectrometric Immunoassay, Therapeutic Monoclonal Antibodies, MSIA Streptavidin D.A.R.T.'s, Anti-IgG-Fc, Ligand Binding Assays, High Resolution/Accurate Mass, Automated Liquid Handling.

Goal:

To demonstrate the effectiveness of analyzing mAbs of different allotypes using Thermo Scientific™ Streptavidin MSIA D.A.R.T.'S with the LB-MSIA™ workflow; a preclinical bioanalytical solution, based on mass spectrometric detection, specific for the bioanalysis of humanized, fully human and chimeric therapeutic monoclonal antibodies.

Introduction:

Presented is a demonstration of the developed Ligand Binding-Mass Spectrometric Immunoassay (LB-MSIA), a universal workflow solution for targeted pre-clinical analysis of therapeutic mAbs, to enable the analyses of therapeutic mAbs of differing allotypes. Historically, ligand binding assays (LBAs) have represented the gold standard for protein quantitation. However, with the increase of biotherapeutic innovation and biological complexity, classic protein analytical techniques such as LBAs are not able to meet the data needs for pharmacokinetics, biotransformation assessment, and antibody functional determination studies. The development of novel antibody therapeutics requires structural information such as variants, sites of glycosylation, post-translation modifications (PTMs) necessary for drug safety, efficacy and stability. Focusing on the enablement of preclinical discovery and development research, the resultant automated and high throughput LB-MSIA combines the robust nature of traditional LBAs with High Resolution/Accurate Mass (HRAM) mass spectrometric detection of intact mAbs. This hybrid bioanalytical workflow is specifically enabled by Streptavidin MSIA D.A.R.T.'S technology; a proprietary product that contains molecular trapping microcolumns covalently derivatized with streptavidin fixed within a pipette tip. When the MSIA D.A.R.T.'S are



coupled with a high affinity reagent, such as biotinylated anti-human IgG Fc affinity ligands, the workflow is able to selectively analyze for fully-human, humanized, and chimeric mAbs spiked within rodent plasma. Below is a demonstration of the selectivity of this LB-MSIA for mAbs of three different allotype subclasses; IgG1-kappa, IgG2-kappa and IgG4-kappa.

This LB-MSIA demonstrated percent coefficients of variation (% CV) and sensitivity akin to a traditional LBA for infliximab (Remicade®), denosumab (Prolia®), natalizumab (Tysabri®) and trastuzumab (Herceptin®). In addition, the assay established the sensitive reproducible detection of intact therapeutic mAb as low as 31.25 ng/mL in mouse plasma.

Materials:

- Thermo Scientific™ Streptavidin MSIA™ D.A.R.T.'S, PN: 991STR12
- Thermo Scientific™ Finnpiptette™ Novus i Multichannel Electronic Pipette, PN: 991SP12
- Alternative High-Through-Put Option: Thermo Scientific™ Versette™ Automated Liquid Handler
- Thermo Scientific™ Finnpiptette™ F1 Adjustable-Volume Pipettes, PN: 4700850
- CaptureSelect™ Biotin Anti-IgG-Fc (Human) Conjugate, PN: 7103262100
- Infliximab (Janssen™ Remicade®)
- Denosumab (Amgen® Prolia®)
- Natalizumab (Biogen® Tysabr®)
- Trastuzumab (Genentech™ Herceptin®)
- Mouse Plasma (K2 EDTA)
- Thermo Scientific™ BupH™ Modified Dulbecco's Phosphate Buffered Saline (PBS) Packs, PN: 28374
- MSIA™ Elution Buffer
- Fisher Chemical™ Optima™ LC/MS Grade Water, PN: W6
- Fisher Chemical™ Optima™ LC/MS Grade Formic Acid, PN: A117
- Fisher Chemical™ Optima™ LC/MS Grade Acetonitrile, PN: A955
- Thermo Scientific™ Nunc™ 500µL 95-Well Plates, Polypropylene, PN: 12-565-368

- Thermo Scientific™ Nunc™ 1.3 and 2.0mL DeepWell Plates with Shared-Wall Technology, 96 DeepWell, Polypropylene, PN: 260252
- Thermo Scientific™ ProSwift™ RP-4H Monolith Column, 1.0 x 250 mm, PN: 066640
- Thermo Scientific™ Vanquish™ UHPLC System
- Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer
- Thermo Scientific™ Xcalibur™ Software, Version 3.0
- Thermo Scientific™ Protein Deconvolution Software, Version 4.0 with the ReSpect™ algorithm

Method

The LB-MSIA workflow for the bioanalysis of therapeutic antibodies may be broken down into five major steps as illustrated in Figure 1. Prior to beginning, a Thermo Scientific™ Novus i electronic pipette was prepared to process 12 samples simultaneously. The Novus i is used to providing the repetitive bidirectional pipetting (aspirating and dispensing cycles) necessary to pass solutions through the microcolumn housed within each of the MSIA D.A.R.T.'S. The Streptavidin MSIA D.A.R.T.'S are first derivatized with a biotin-conjugated anti-IgG Fc, an affinity ligand that specifically binds to the Fc portion of all four human IgG subclasses. The next step is to assay for the fully human, humanized or chimeric therapeutic monoclonal antibody from rodent plasma samples by incubating the samples with the anti-IgG-Fc-derivatized Streptavidin MSIA D.A.R.T.'S. The affinity bound mAb is subsequently released from the microcolumn by treatment with the elution buffer. The ensuing eluate containing the therapeutic mAb is then analyzed using LC-MS (HRAM). Utilizing Thermo Scientific's™ Xcalibur™ (Version 2.2) and Protein Deconvolution (Version 3.0) software the MS data is deconvolved to provide high content qualitative data.

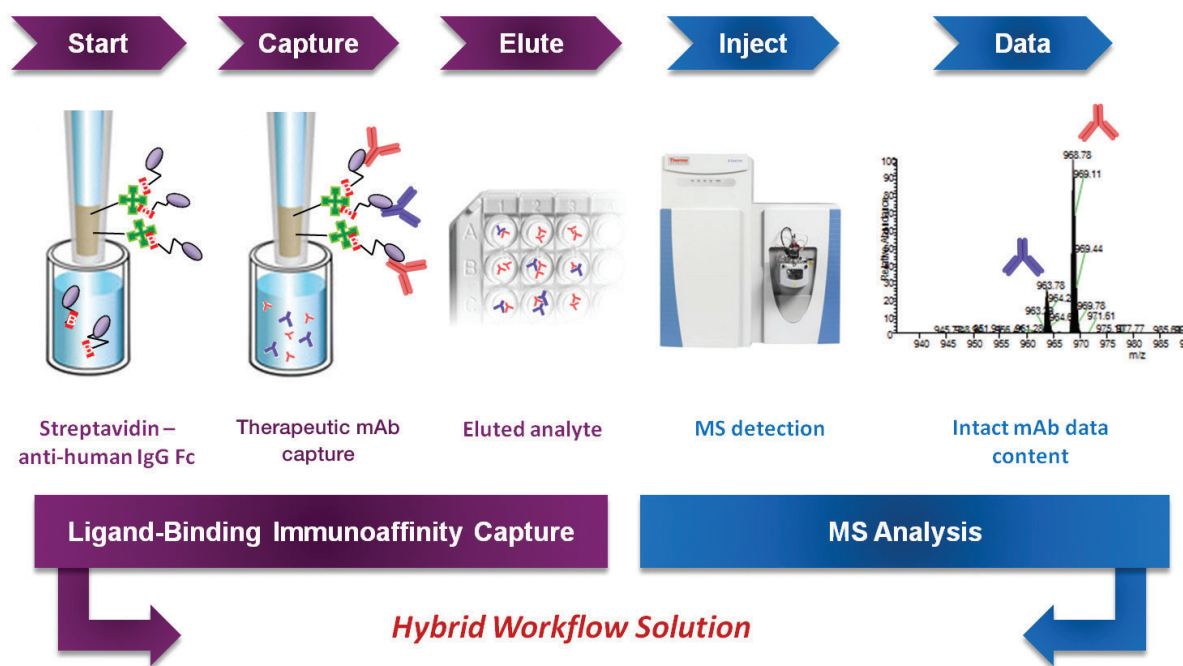


Figure 1— A schematic showing the five major steps of the LB-MSIA workflow

Pre-Analytical

Derivatization of Streptavidin MSIA D.A.R.T.'S with Affinity Ligand

To enable the Streptavidin MSIA D.A.R.T.'S to have a specific affinity for humanized, fully human and chimeric mAbs, each of the streptavidin derivatized microcolumns were loaded with 125 µL of 4 µg/mL CaptureSelect™ biotin anti-IgG-Fc (Human) conjugate, a single domain antibody (Life Technologies), prepared in PBS (BupH™ Modified Dulbecco's PBS). This was accomplished by following the steps provided in Table 1 utilizing a Thermo Scientific™ Novus i electronic pipette equipped with Streptavidin MSIA D.A.R.T.'S.

Assay Step		Assay Solution	Total Well Volume (μL)	Asp/ Disp Volume (μL)	Asp/ Disp Cycles	Speed
1	Buffer Pre-Rinse	PBS	200	150	10x	4
2	Immobilization of anti-IgG-Fc	Biotin anti-IgG Fc conjugate antibody	125	70	500x	1
3	Buffer Rinse	PBS	200	150	10x	4
4	Buffer Rinse	PBS	200	150	10x	4

Table 1 – Derivatization of Streptavidin MSIA D.A.R.T.'S with biotinylated anti-human IgG Fc: Novus i protocol in descending order

Sample Preparation

All samples prepared consisted of 200 µL of mouse plasma supplemented with varying concentrations of one of four therapeutic mAbs (infliximab, denosumab, natalizumab or trastuzumab). The dosing curve ranges utilized for each mAb are listed in the Table 2.

Therapeutic mAb	Dynamic Range (µg/mL)
Infliximab (Remicade®)	62.5–4000
Denosumab (Prolia®)	500–64,000
Natalizumab (Tysabri®)	31.25–8000
Trastuzumab (Herceptin®)	31.25–8000

Table 2 – mAb dosing curve ranges for LB-MSIA sample preparation

Prior to incubation of the samples with the anti-IgG-Fc-derivatized Streptavidin MSIA D.A.R.T.'S each sample was further diluted with 200 µL PBS. Using the Novus i, the following steps outlined in Table 3 were performed to capture the therapeutic mAbs from the samples.

Assay Step		Assay Solution	Total Well Volume (µL)	Asp/ Disp Volume (µL)	Asp/ Disp Cycles	Speed
1	Therapeutic mAb Capture*	Sample solution	400	300	500x	1
2	Buffer Rinse	PBS	200	150	10x	4
3	Buffer Rinse	PBS	200	150	10x	4
4	Water Rinse	Water	200	150	10x	4
5	Water Rinse	Water	200	150	10x	4

*by Anti-IgG-Fc MSIA D.A.R.T.'S

Table 3 – Therapeutic mAb Capture: Novus i protocol in descending order

Sample Elution

Following the selective capture of the therapeutic mAb with the anti-IgG-Fc-derivatized Streptavidin MSIA D.A.R.T.'S, each device was treated with 100 µL of the MSIA™ Elution Buffer liberating the mAb. Reference Table 4 for the specifics of the repetitive pipetting used to elute the captured mAb from the D.A.R.T.'S. The intact mAb was then analyzed by LC-MS (HRAM).

Assay Step		Assay Solution	Total Well Volume (μL)	Asp/Disp Volume (μL)	Asp/Disp Cycles	Speed
1	Elution	Elution Buffer	100	40	20x	48

Table 4 – Novus i protocol for eluting affinity captured therapeutic mAb from anti-human IgG Fc derivatized D.A.R.T.'S

Analytical-Detection

Liquid Chromatography

The affinity-purified therapeutic mAb eluates were separated on a Thermo Scientific™ Dionex™ UltiMate® 3000 RSLC (Rapid Separation Liquid Chromatography) system utilizing a Thermo Scientific™ ProSwift™ RP-4H (1 x 250 mm) column heated to 60 °C. Separation was performed utilizing a gradient of 10%-38% B (B: acetonitrile, 0.2% formic acid) with a flow rate of 200 uL/min over 12 minutes.

Mass Spectrometry

For all samples, full-scan MS data were acquired over the range of m/z 2000-3400 m/z in positive-ion mode on a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer with a resolving power of 17,500 (FWHM) at m/z 200 and the AGC (Automatic Gain Control) set to a target value of 3.00E6.

Data Analysis

All LC-MS raw data was collected using Thermo Scientific's Xcalibur software, Version 2.2. From the raw MS data an extracted ion chromatogram was generated for the five most abundant charge states of the intact therapeutic mAbs, which were then integrated to obtain the AUC (Area Under the Curve) value for each sample analyzed.

Further characterization of the mAbs, specifically in reference to the presence of glycosylation, was obtained from processing the MS raw data using Thermo Scientific's™ Protein Deconvolution™ Software Version 3.0 utilizing the ReSpect™ algorithm.

Results and Discussion

The MSIA workflow provides a unique push button and automated solution for therapeutic mAb bioanalytics for a broad variety of therapeutic mAbs with differing allotype subclasses. The allotype subclasses of the four therapeutic mAbs used in this study are presented in Table 5.

mAb Analyzed	Construct	Allotype Subclass
Trastuzumab (Herceptin®)	Fully Human	IgG1-kappa
Infliximab (Remicade®)	Chimeric	IgG1-kappa
Denosumab (Prolia®)	Fully Human	IgG1-kappa
Natalizumab (Tysabri®)	Humanized	IgG4-kappa

Table 5 – Description of therapeutic mAbs with differing constructs and allotype subclasses used to test the LB-MSIA™ workflow

By combining the performance characteristics of traditional ligand binding assays with the benefits of HRAM MS detection, high value data content is produced that is highly sensitive, accurate, and reproducible.

Dynamic Range Comparison of Five Therapeutic Monoclonal Antibodies of Differing Allotypes

To test the sensitivity of the LB-MSIA workflow for each of the four therapeutic mAbs of differing allotypes from mouse plasma, samples were run producing a plot akin to a dosing curve represented in Figure 2. The therapeutic mAbs deconvolved masses are represented in Table 6. For each sample the AUC from the extracted ion chromatogram for the top five charge states were summed and the average for each concentration was then used to generate the plots in Figure 2. The assay achieved a linear dynamic range for each mAb analyzed with CVs of the triplicate control samples within the acceptable range for traditional ligand binding assays represented in Table 6.

mAb Analyzed	Dynamic Range (µg/mL)	Control Concentration (ng/mL)	Control CVs
Trastuzumab (Herceptin®)	31.25-8000	2500	1.76%
Infliximab (Remicade®)	62.5-4000	1500	1.37%
Denosumab (Prolia®)	500-64,000	10,000	1.82%
Natalizumab (Tysabri®)	31.25-8000	500	1.17%

Table 6 – Summary of the control samples studied for each of the four therapeutic mAbs. Control samples also utilized 200 µL of mouse plasma.

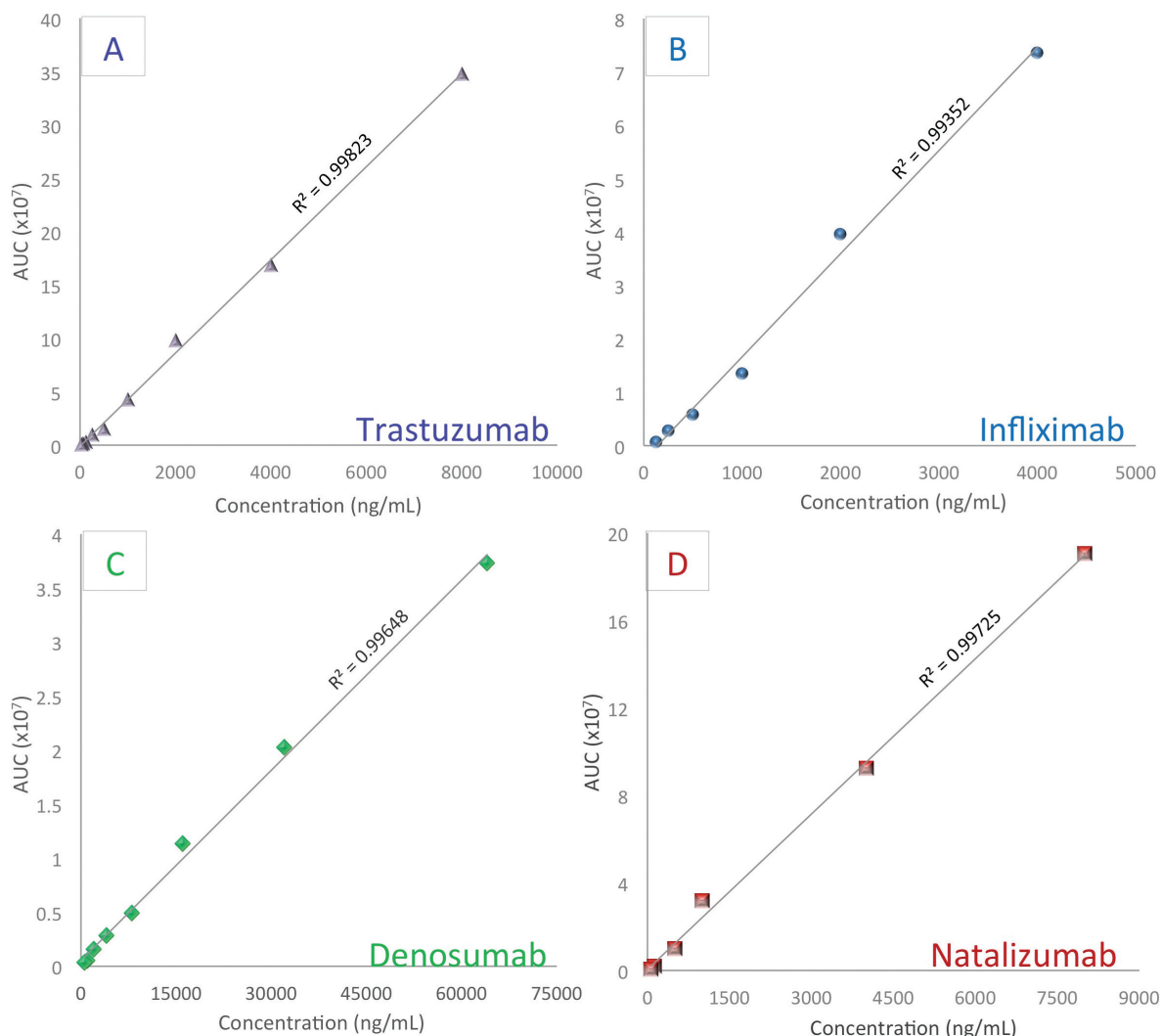


Figure 2 – Results of the intact analysis of four therapeutic mAbs analyzed with the LB-MSIA workflow that spanned the following dynamic ranges:
 A) Dynamic range of trastuzumab: 31.25-8000 ng/mL
 B) Dynamic range of infliximab: 62.5-4000 ng/mL
 C) Dynamic range of denosumab: 500-64,000 ng/mL
 D) Dynamic range of natalizumab: 31.25-8000 ng/mL

Conclusion

The LB-MSIA provided a highly sensitive and robust method for the generation of high value data content for the bioanalysis of each of the fully human, humanized and chimeric therapeutic antibodies with differing allotypes. The high selectivity of the CaptureSelect™ biotin anti-IgG-Fc (human) conjugate combined with the molecular trapping technology of the MSIA D.A.R.T.'S creates an ideal scenario to assay low abundant (ng/mL) intact human therapeutic mAbs from rodent plasma. As a hybrid approach, the use of the Q Exactive™ for HRAM detection helps provide additional data content over other developing triple quadrupole methods that are reliant on peptide analysis. As shown, the combined benefits of the LB-MSIA enable the characterization of multiple intact mAbs over a wide dynamic range while maintaining coefficients of variation of <2% for control samples.

Ordering Information

MSIA D.A.R.T.'S for Immunoaffinity Capture		
Compatible with the Thermo Scientific™ Versette™ Automated Liquid Handler and Thermo Scientific™ Finnpiptette® Novus i Multichannel Electronic Pipette		
Cat. No.	Description	Packaging
991CUS02	300µl MSIA D.A.R.T.'S, Custom	Pack of 96 units
991PRT11	300µl MSIA D.A.R.T.'S, Protein A	Pack of 96 units
991PRT12	300µl MSIA D.A.R.T.'S, Protein A	Pack of 24 units
991PRT13	300µl MSIA D.A.R.T.'S, Protein G	Pack of 96 units
991PRT14	300µl MSIA D.A.R.T.'S, Protein G	Pack of 24 units
991PRT15	300µl MSIA D.A.R.T.'S, Protein A/G	Pack of 96 units
991PRT16	300µl MSIA D.A.R.T.'S, Protein A/G	Pack of 24 units
991STR11	300µl MSIA D.A.R.T.'S, Streptavidin	Pack of 96 units
991STR12	300µl MSIA D.A.R.T.'S, Streptavidin	Pack of 24 units
991001096	300µl MSIA D.A.R.T.'S, Insulin	Pack of 96 units
991001024	300µl MSIA D.A.R.T.'S, Insulin	Pack of 24 units
991R	300 µL MSIA D.A.R.T.'S, Reloadable Rack	1 reloadable rack, D.A.R.T.'S are not included
MSIA Streptavidin-EVO for Immunoaffinity Capture		
Compatible with Tecan™ Freedom EVO® 150 Liquid Handling Robotic Platform equipped with a MCA96 head		
Cat. No.	Description	Packaging
992STR96	500µl MSIA Streptavidin EVO microcolumns	Pack of 96 units
Automated Liquid Handling Platform		
Cat. No.	Description	
650 – MSIA	MSIA Versette Automated Liquid Handler	
Multichannel Pipettes and Pipette Stand		
Cat. No.	Description	Packaging
991S	Finnpiptette Novus i Adjustable Pipette Stand	1 pipette stand
991SP12	Finnpiptette Novus i Electronic 12-Channel Pipette, 30 – 300µl and Pipette Stand	1 pipette and 1 pipette stand
Liquid Chromatography		
Cat. No.	Description	
	Thermo Scientific™ Dionex™ UltiMate™ 3000 UHPLC System	
	Thermo Scientific™ Vanquish™ UHPLC System	
066640	ProSwift RP-4H Monolith Column, 1.0 x 250 mm	
Mass Spectrometry and Software		
Description		
Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer		
Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer		
Thermo Scientific™ Pinpoint Software		
Thermo Scientific™ Xcalibur™ Software		
Thermo Scientific™ Protein Deconvolution Software, Version 4.0 with the ReSpec™ algorithm		

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