Introduction to the Mass Spectrometric Immunoassay workflow (MSIA™) for Bioanalysis of large Biomolecules or Biomarker studies by LC-MS

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What is Mass Spectrometric Immunoassay (MSIA)?
Mass Spectrometric Immunoassay (MSIA)

**Comprehensive MSIA Workflow**

1. **Start**
   - Immobilized Affinity ligand

2. **Select**
   - Target selection

3. **Elute**
   - LC-MS-ready samples

4. **Inject**
   - LC-MS detection

5. **Analyse**
   - Qualitative & Quantitative data

Sample Preparation and Liquid Handling

Detection and Analysis
Affinity Technology
Thermo Scientific Affinity Microcolumn Technology

**Microcolumn** – Derivitised fused-silica medium.

- MSIA D.A.R.T’s- Thermo Scientific liquid handling
- New: MSIA Streptavin EVO- Tecan MCA 96 head

**Sample flows up & down through the microcolumn enabling analyte purification by the affinity ligand**

1. Microcolumn is fixed
   - No particulate loss in handling or into eluate

2. Consistent amount of affinity ligand on microcolumn surface
   - Reproducible assays
   - No aliquoting

3. Low Background
   - High signal to noise

4. Microfluidic channels force molecular interactions
   - Reduces assay time

5. Sample volume independent

6. Microcolumn resistant to compression unlike resin-based support
Workflow
MSIA DART’s- Disposable Automation Research Tips (D.A.R.T’s)
MSIA D.A.R.T’s: Four Options

**MSIA Protein A, G & A/G:**
Flexible platform for assay development with diverse antibodies

**MSIA Streptavidin:**
Tools for developing robust assays with biotinylated affinity ligands
Two workflow options for different target analytes.

**MSIA Insulin:**
Assay platform for affinity purification of insulin analogs from complex matrices for simultaneous qualitative & quantitative analysis by LC-MS

**MSIA Custom:**
Tool for analytical affinity purification.
Affinity ligand is directly coupled to the microcolumn surface.
MSIA D.A.R.T’s: Liquid Handling

Novus I Automated Pipettor and Stand

Methods development of 1-12 samples

Versette Automated Liquid Handling System

Compact size saving bench space:
2-level deck configuration with 6 deck positions

Increased throughput:
Run 96 samples simultaneously.

Simple software
New Product: MSIA Streptavidin-EVO
• Uses unique Streptavidin linked monolithic micro columns housed within Tecan MCA 96 tip format

• Specifically formatted for the MCA 96 liquid handling arm for high throughput applications.

• High binding capacity:
  • Immobilized Streptavidin: 10 µg/micro column
  • Biotinylated Ab: 7.5 µg/micro column
  • Extended assay dynamic range
  • Improved signal
• MSIA Streptavidin-EVO uses standard Tecan Freedom EVO commands
  Addressed using the mix command

• Assays utilize the different stage positions for each incubation and wash step

*Easy adoption*
Example Workflow for Tecan EVO Platform

MSIA Workflow (biological matrix to data)

Start
  - Streptavidin – Capture Select

Capture
  - Therapeutic mAB Capture

Elute
  - Eluted analyte

Analytical - Detection
  - SDS-PAGE/Western blot
  - UV/Vis
  - Mass Spectrometry

Pre-Analytical (fully-automated)

Inject

Data

Complete solution for large molecule bio-analysis
General Application Overview
Biotherapeutics

- Ligand-binding mass spectrometry to study biotransformation of fusion protein drugs and guide immunoassay development: strategic approach and application to **peptibodies** targeting the thrombopoietin receptor.
- Development of a Potential High-Throughput Workflow to Characterize Sites of Bioconjugation by Immuno-Affinity Capture

Biomarkers

- Screening **protein isoforms** predictive for **cancer** using immuno-affinity capture and fast LC-MS in PRM mode
- A semi-automated mass spectrometric immunoassay coupled to selected reaction monitoring (MSIA–SRM) reveals novel relationships between circulating **PCSK9** and metabolic phenotypes in patient cohorts

Toxicology/food safety/Anti-doping

- Monolith immuno-affinity enrichment liquid chromatography tandem mass spectrometry for quantitative protein analysis of **recombinant bovine somatotropin** in serum
- Qualitative identification of **growth hormone-releasing hormones** in human plasma by means of immunoaffinity purification and LC-HRMS/MS

Exosomes

- Antibody-coupled monolithic silica microtips for high throughput profiling of circulating **exosomes**
European Bioanalytical Forum

- Lieve Dillen, Janssen R&D: Challenges with a LCMS method for quantification of an oligonucleotide

- Rand Jenkins, PPD: Direct Bioanalysis of ADCs using Affinity Capture-LC-HR/AMS Techniques for Characterization and Quantification—a Progress Update

- Michael Blackburn, Covance: How low can you go: Driving down limits of quantitation for peptide biomolecules by hybrid IA-LC/MS

IMSC

- William Van Dongen, TNO Triskelion: Bioanalytical LC-MS of Therapeutic mAbs Sensitivity and selectivity optimization strategies involving MSIA, EASY-Spray and Q Exactive
Thank You for Listening
More to come...