



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

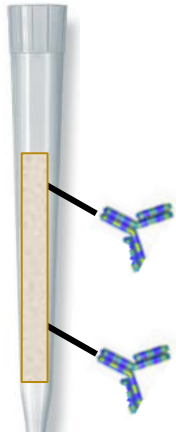
**Ravindra Chaudhari, PhD, Field Application Specialist**

- **What is MSIA?**
- **Affinity Technology**
- **Workflow**
- **MSIA DART's- Disposable Automation Research Tips**
- **New: MSIA Streptavidin EVO**
- **General Application Overview**

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

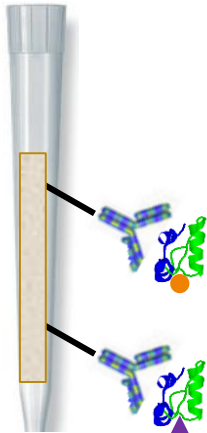
# Mass Spectrometric Immunoassay (MSIA)

Start



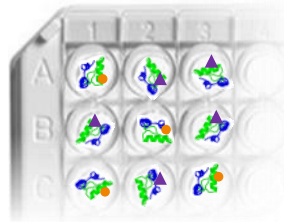
Immobilized  
Affinity ligand

Select



Target  
selection

Elute



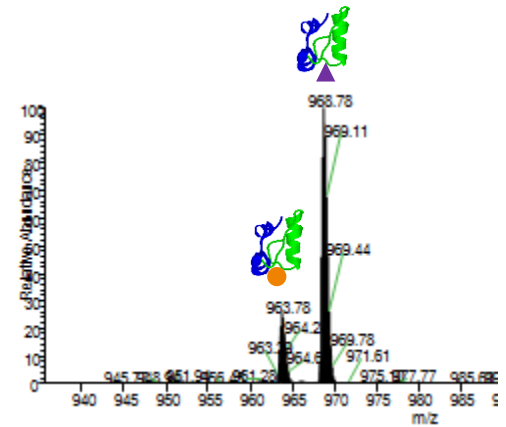
LC-MS-ready  
samples

Inject



LC-MS  
detection

Analyse



Qualitative &  
Quantitative data

Sample Preparation and Liquid Handling

Detection and Analysis

**Comprehensive MSIA Workflow**

# Affinity Technology

# Thermo Scientific Affinity Microcolumn Technology



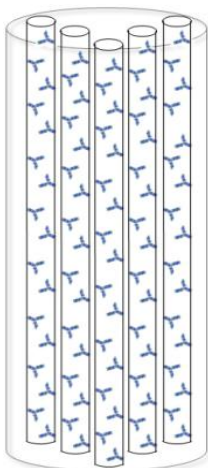
Plastic pipette tips containing MSIA microcolumn

- MSIA D.A.R.T's- Thermo Scientific liquid handling

OR

- New: MSIA Streptavin EVO- Tecan MCA 96 head

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



**Microfluidic Channels** – contain immobilized affinity ligands.

*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. Micro column 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

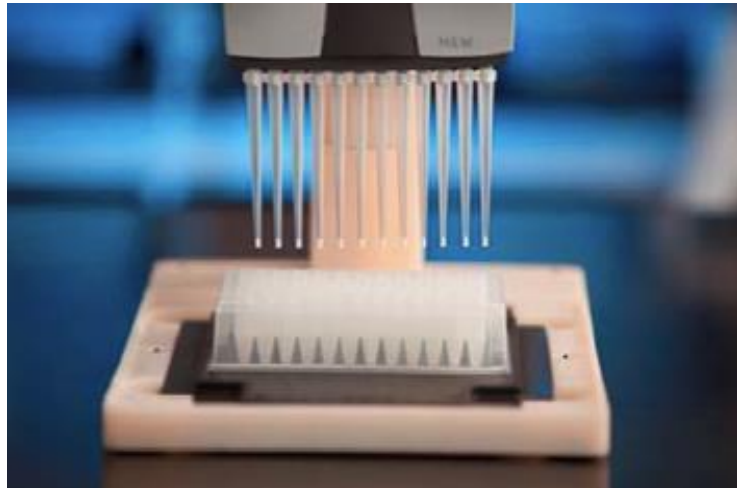
## **MSIA Custom:**

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.

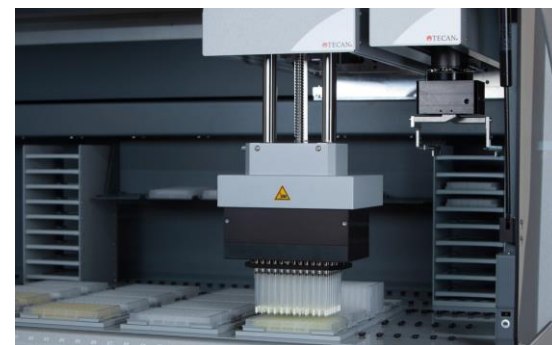
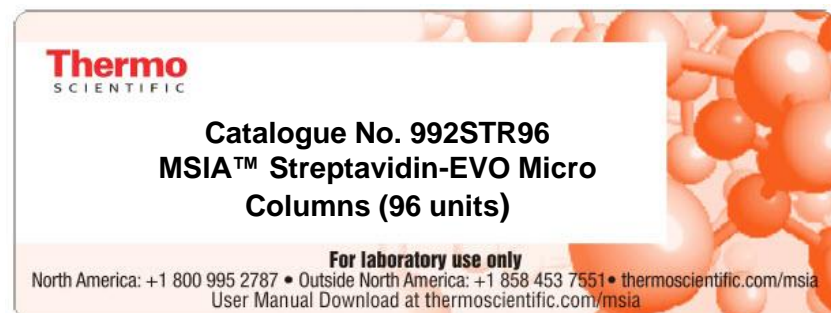
Simple software



## **New Product: MSIA Streptavidin-EVO**

# 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: Utilisation

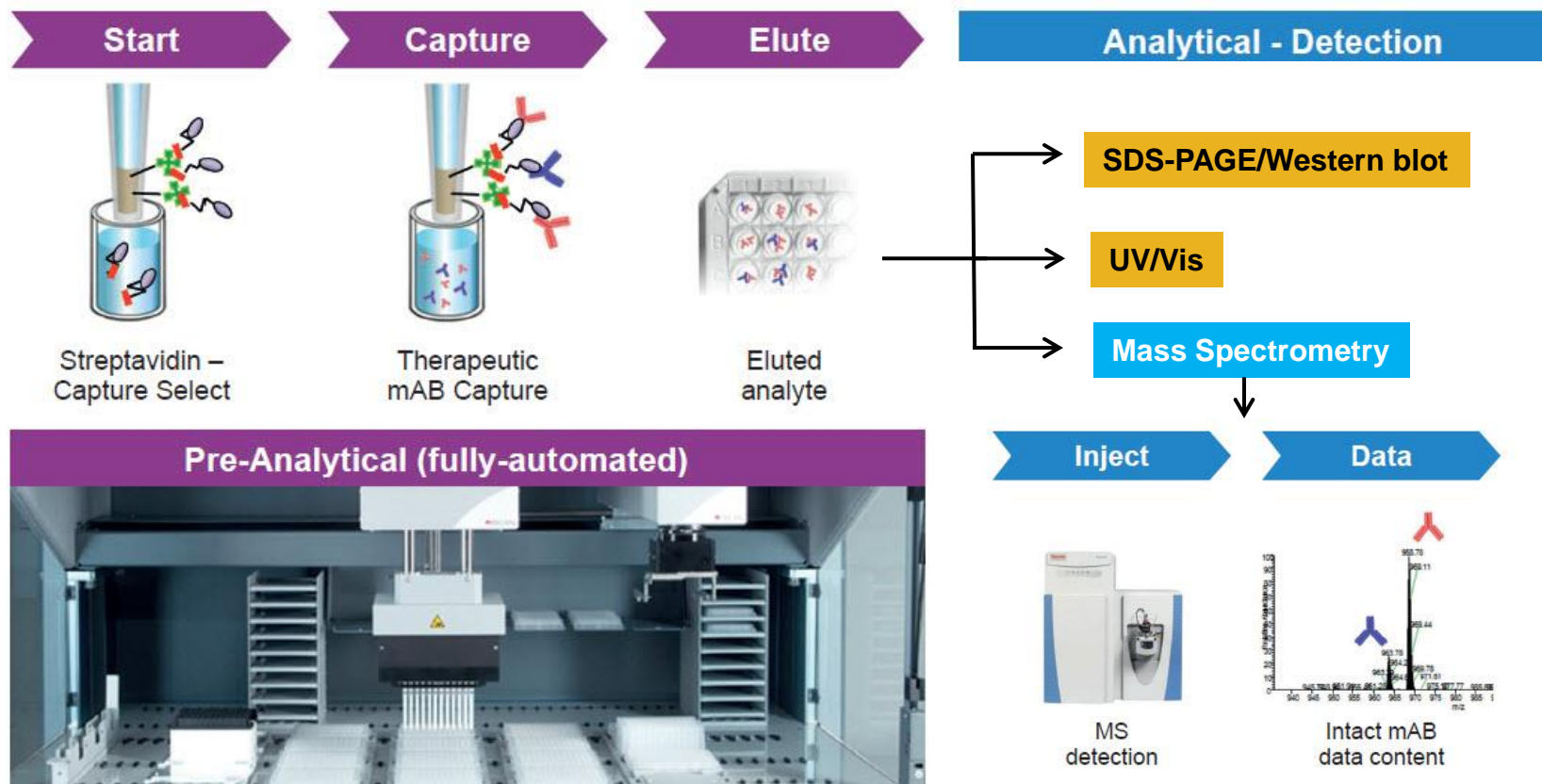


- **MSIA Streptavidin-EVO uses standard Tecan Freedom EVO commands**  
Addressed using the mix command
- **Assays utilize the different stage positions for each incubation and wash step**

*Easy adoption*

# Example Workflow for Tecan EVO Platform

## MSIA Workflow (biological matrix to data)



# General Application Overview



# 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...**