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A Rapid and Reproducible Immuno-MS Platform from Sample Collection to Quantitation of IgG

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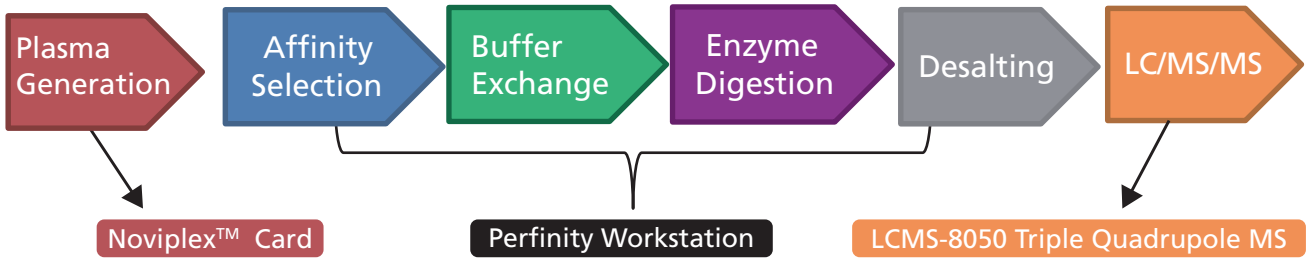
Novel Aspect

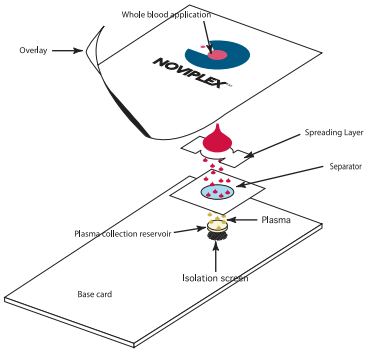
Using rapid, automated processing, coupled to the speed and sensitivity of the LCMS-8050 allows for improved analysis of Immunoglobulin G.

Introduction

Dried blood spot analysis (DBS) has provided clinical laboratories a simple method to collect, store and transport samples for a wide variety of analyses. However, sample stability, hematocrit effects and inconsistent spotting techniques have limited the ability for wide spread adoption in clinical applications. Dried plasma spots (DPS) offer new opportunities by providing stable samples that avoid variability caused by the hematocrit. This presentation focuses on an ultra-fast-immuno-MS platform that combines next generation plasma separator cards (Novilytic L.L.C., North Webster, IN) with fully automated immuno-affinity enrichment and rapid digestion as an upfront sample preparation strategy for mass spectrometric analysis of immunoglobulins.

Sample Workflow





Rapid plasma extraction technology from whole blood (~ 18 minutes)
- 2.5 uL of plasma collected (3 min)
- Air dry for 15 minutes
- Extract plamsa disc for analysis

Automates and integrates key proteomic workflow steps:
- Affinity Selection (15 min)
- Trypsin digestion (1-8 min)
- Online Desalting
- Reversed phase LC

Exceptional reproducibility (CV less than 10%)

- Ultrafast MRM methods
- Up to 555 MRM transitions per run
- Heated electrospray source
- Scan speeds up to 30,000 u/sec
- Polarity switching 5 msec

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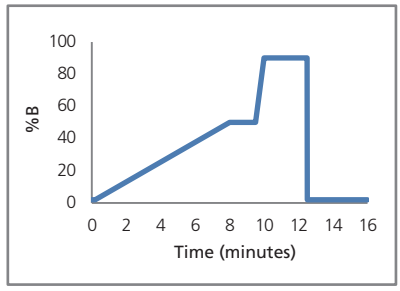
Methods

IgG was weighed out and then diluted in 500 µL of 0.5% BSA solution. Approximately15 uL of IgG standard was spiked into mouse whole blood and processed using the Noviplex card. The resulting plasma collection disc was extracted with 30 uL of buffer and each sample was reduced and alkylated to yield a total sample volume of 100 uL. IgG standards and QC samples were directly injected onto the Perfinity-LCMS-8050 platform for affinity pulldown with a Protein G column followed by trypsin digestion and LC/MS/MS analysis.

Level	Conc. (µg/mL)	Amount on column (µg)	Amount on column (pmol)
1	465	34.88	581.25
2	315	23.63	393.75
3	142.5	10.69	178.13
4	127.5	9.56	159.37
5	102	7.65	127.50
6	60	4.50	75.00
7	22.5	1.69	28.12

IgG concentrations for calibration levels.

Time (min)	%B
0	2
0.2	2
8	50
9.5	50
10	90
12.5	90
12.51	2
16	2



LCMS gradient conditions.

Compound Name	Transitions	+/-	Q1 Rod Bias (V)	CE (V)	Q3 Rod Bias (V)
TTPPVLDSDGSFFLYSK	937.70>836.25	+	-27	-28	-26
	937.70>723.95	+	-27	-30	-22
VVSVLTVLHQDWLNGK	603.70>805.7	+	-22	-16	-13

MRM transitions on LCMS-8050 for two IgG peptides monitored.

Noviplex Cards

(1)

(2)

(3)

(4)

Approximately 50 uL of the spiked whole blood was pipetted onto the Noviplex card test area (1). The spot was allowed to dry for 3 minutes (2). The top layer of the card was then peeled back (3) to reveal the plamsa collection disc. The plasma collection disc was allowed to dry for an additional 15 minutes. Once the disc was dry (4), it was placed into an eppendorf tube for solvent extraction.

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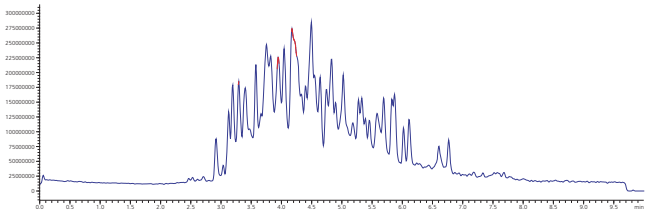
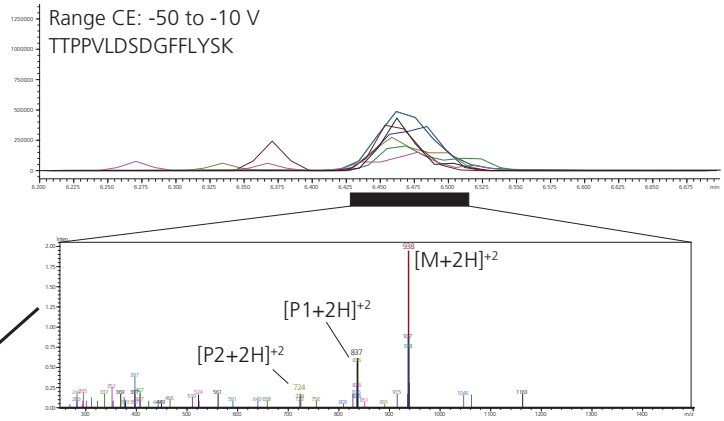
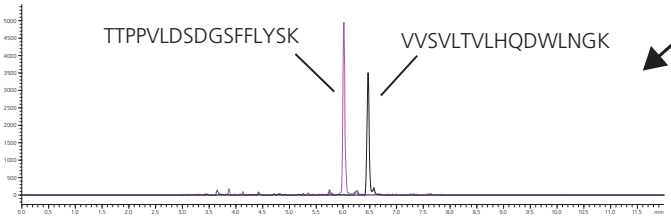
Results - Chromatograms



Total Ion Chromatogram for IgG

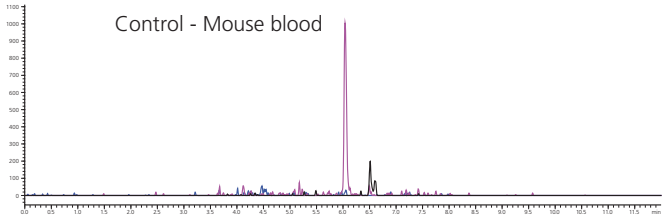
Optimization of Collision Energies for peptides of interest

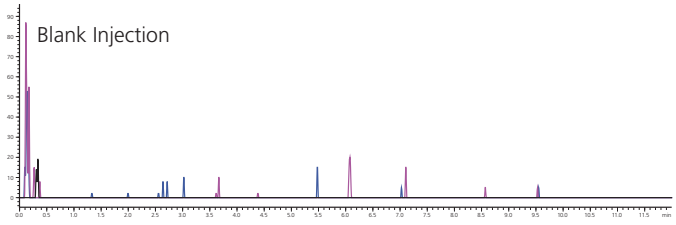




MRM Chromatogram for Level 4 standard of spiked IgG in whole blood.

Carryover Assessment





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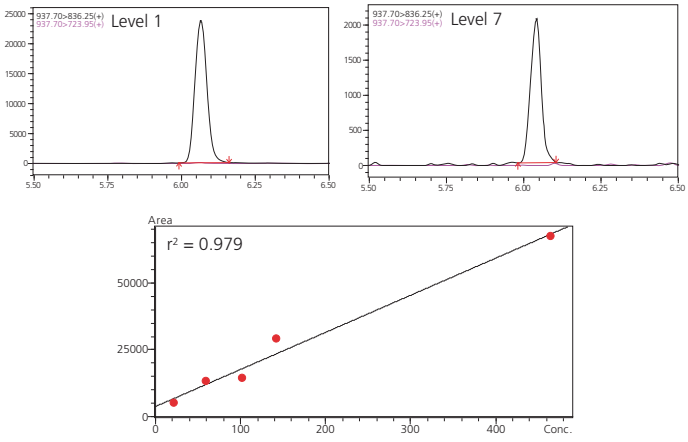
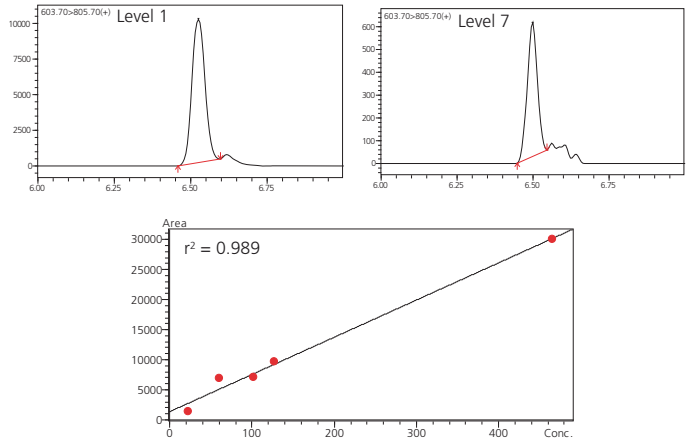
Results - Calibration Curves

Calibration Curve and MS Chromatograms

TTPPVLDSDGSFFLYSK



VVSVLTVLHQDWLNGK



Results - Tables and Replicates

QC data and Calculations for IgG Peptides

VVSVLTVLHQDWLNGK					
Sample	Ret. Time	Area	Calc. Conc.	Std. Conc.	% Accuracy
QC 1	6.49	32,492	502.804	465	108.1
QC 2	6.516	11,726	167.189	142.5	117.3
QC 3	6.514	8,507	115.155	102	112.9
QC 4	6.492	2,727	21.745	22.5	96.6

TTPPVLDSDGSFFLYSK					
Sample	Ret. Time	Area	Calc. Conc.	Std. Conc.	% Accuracy
QC 1	6.029	61,525	416.447	465	89.6
QC 2	6.052	25,355	155.568	142.5	109.2
QC 3	6.047	16,900	94.58	102	92.7
QC 4	6.029	6,502	19.587	22.5	87.1

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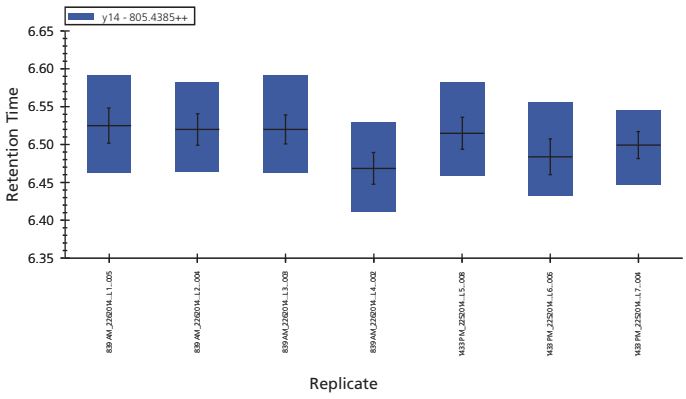
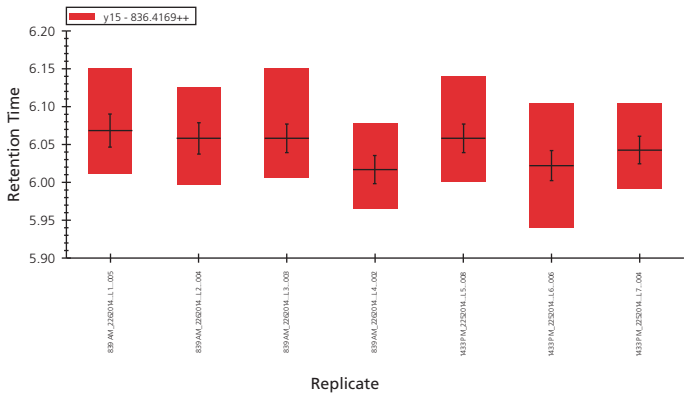
Skyline Data - Retention Time Replicates

VVSVLTVLHQDWLNGK



TTPPVLDSDGSFFLYSK



Integration of Skyline Software into LabSolutions allows for further interrogation of data. Here are representative figures showing the retention time reproducibility for each peptide monitored during the analysis.

Conclusions

Combining the sample collection technique of next generation plasma separator Noviplex cards for quick plamsa collection from whole blood, with the automated affinity selection and trypsin digestion of the Perfinity workstation coupled to LCMS-8050, provides a very rapid and reproducible Immuno-MS platform for quantitation of IgG peptides. Furthermore, this rapid immuno-MS platform can be applied to many other peptide/protein applications.

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