Development of ELISA plate-based immunocapture for LC/MS/MS analysis of therapeutic proteins

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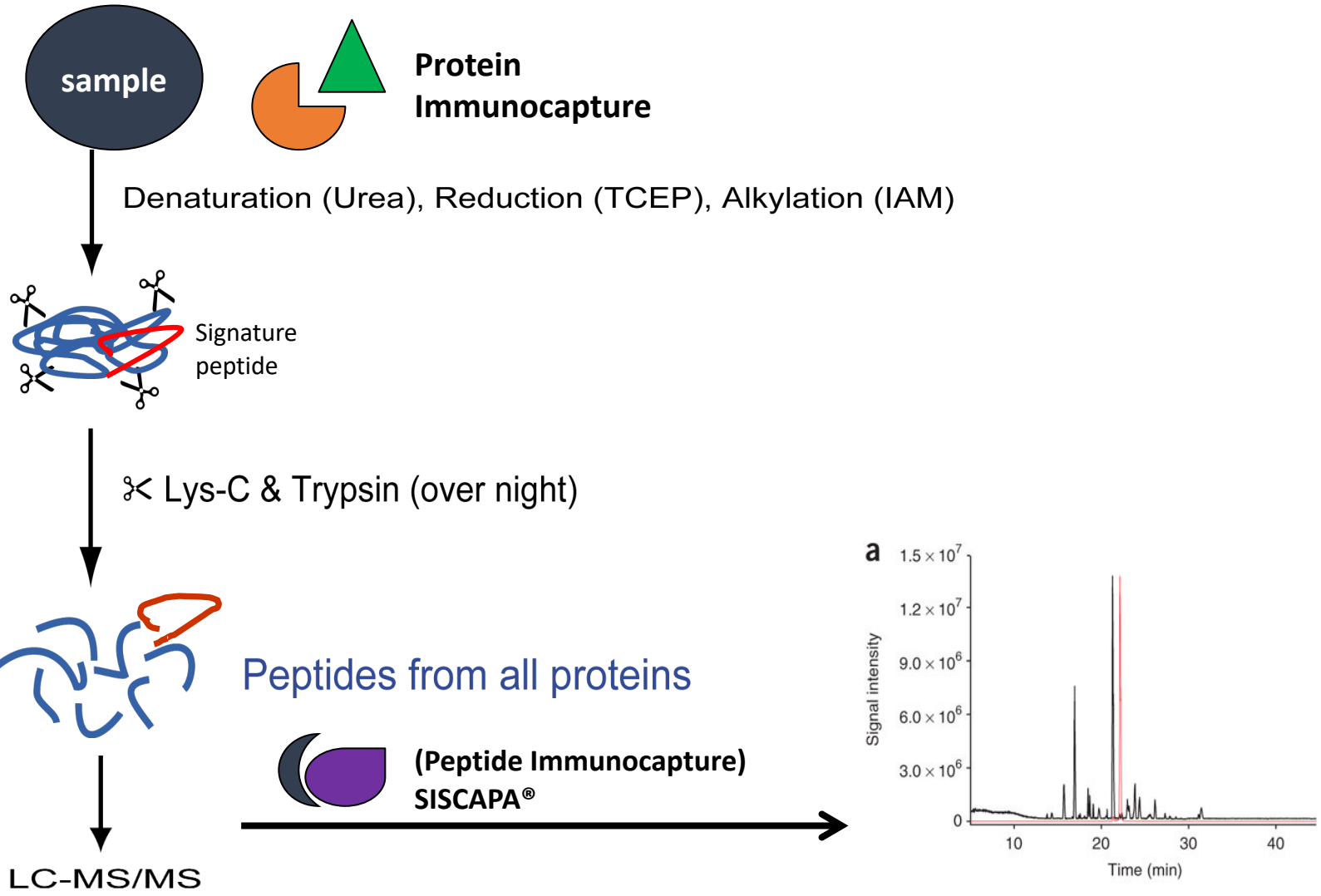
Outlines

- Introduction
- Hybrid Assay Development
- Results
- Summary

Quantitation of Biotherapeutics by Proteolytic digestion -LC/MS



Quantitation of Biotherapeutics by LC/MS

Advantages

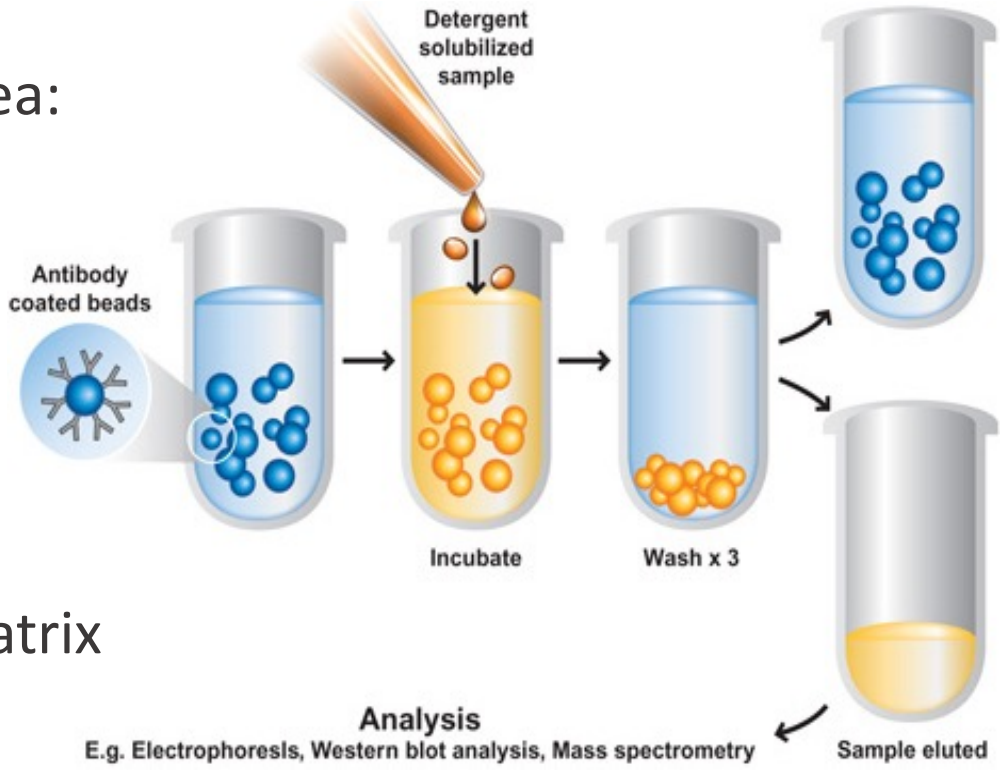
- Wide dynamic range: up to 4 orders of magnitude
- Multiplexing and multiple species/matrices
- High selectivity and specificity
- Less strict requirements for immunocapture reagents due to LC/MS selectivity
- Fast method development: 3-4 weeks
- Able to monitor biomodification

Limitations

- May not be suitable to measure free drug (e.g. antibody drug)
- Throughput
- Cost

Immunocapture using magnetic beads

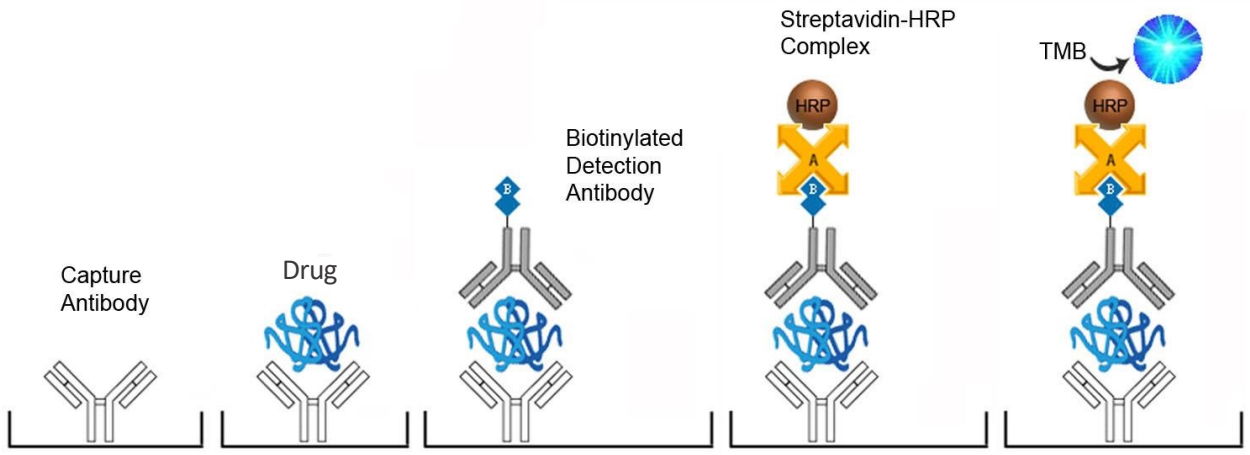
- Advantages:
  - Considerable surface area: high binding capacity
  - Excellent consistency
  - Flexible
  - Easy to handle
- Limitations:
  - Non-specific binding: matrix interference
  - Cost



Source: http://www.mitosciences.com/Immunocapture.jpg

Quantitation of Biotherapeutics using ELISA

A typical Sandwich ELISA



Source: https://lsbio-7d62.kxcdn.com/image2/mouse-agrn-agrin-elisa-kit-sandwich-elisa-ls-f16819/255792_1927613.jpg

ELISA for quantitation of biotherapeutics

- Advantages:
  - High throughput
  - Low cost
  - High sensitivity
  - Automation
- Limitations:
  - More strict requirement for quality of capture and detection reagent
  - Endogenous interference
  - Usually narrow dynamic range

ELISA immunocapture platform for LC/MS

Bioanalysis. 2015;7(3):307-18. doi: 10.4155/bio.14.250.

ELISA microplate: a viable immunocapture platform over magnetic beads for immunoaffinity-LC-MS/MS quantitation of protein therapeutics?

Yang W[1], Kernstock R, Simmons N, Alak A.

Bioanalysis. 2016 Oct;8(20):2103-14. doi: 10.4155/bio-2016-0180. Epub 2016 Sep 9.

A whole-molecule immunocapture LC-MS approach for the in vivo quantitation of biotherapeutics.

Kellie JF[1], Kehler JR[1], Mencken TJ[1], Snell RJ[2], Hottenstein CS[1].

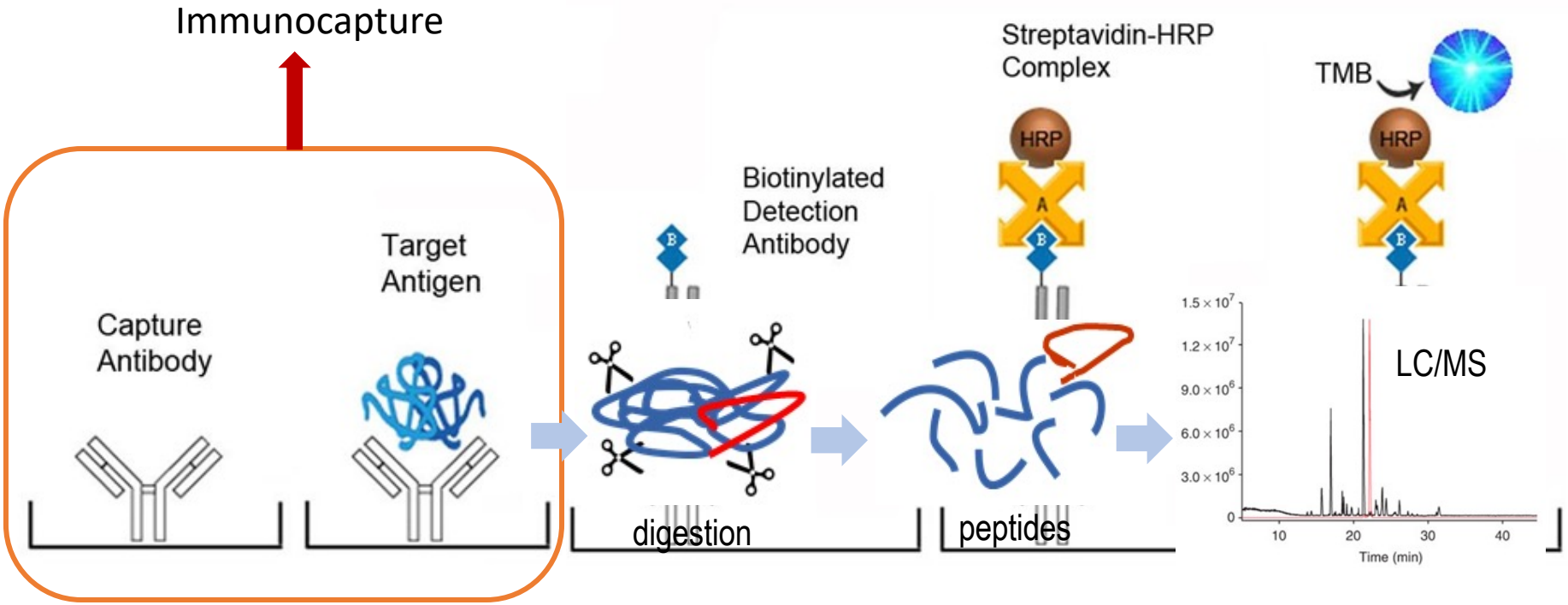
Bioanalysis (2018) 10(17), 1427–1438

Development of an ELISA–LC–MS hybrid assay for quantification of biotherapeutics

Linzhi Chen*[,1], Siyu Liu[1,2], Shirin Pagels[1], Caitlin Quatrano[3], Dave Roos[1], Elsy Philip[1], Henry Zhao[1] & Hongbin Yu[1]

ELISA plate for immunocapture-LC/MS

Sandwich ELISA



Source: https://lsbio-7d62.kxcdn.com/image2/mouse-agrn-agrin-elisa-kit-sandwich-elisa-ls-f16819/255792_1927613.jpg

ELISA plate immunocapture platform

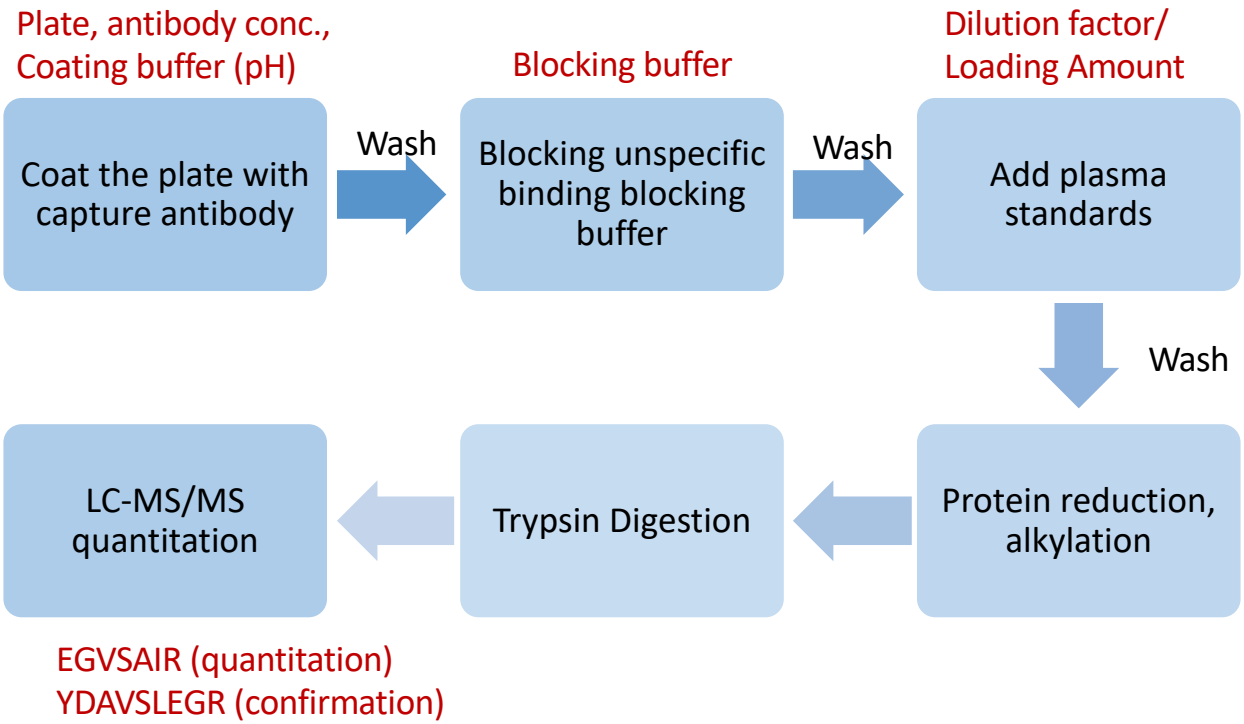
- Advantages:
  - Low non-specific binding (ELISA plate)
  - Low endogenous interference (LC/MS selectivity and specificity)
  - Lower cost (no beads)
  - Automation

- Limitations:
  - Capture capacity (ELISA plate)
  - Dynamic range (ELISA plate)

Hybrid Assay Development

- Parameters for test and optimization
  - Biotherapeutic
    - 80 kDa protein
  - Matrix
    - cyno plasma
  - ELISA plates
    - Pierce™ streptavidin coated high capacity plate
    - GE Streptavidin
    - R&D streptavidin
    - Sigma Streptavidin
    - Nunc MaxiSorp™ flat-bottom plate
    - Fisherbrand™ 96-well polystyrene plate (surface unmodified)
    - Pierce™ maleic anhydride activated plate
  - Capture antibody amount
    - 1 µg, 5 µg, 10 µg, 20 µg
  - Sample amount
    - 100 µl, 50 µL, 33 µL, 20 µL
  - Reagent
    - PBS (pH7.2), sodium bicarbonate (pH9)
    - Blocking Casein, PBS+1%BSA
- Compare with magnetic beads-based immunocapture-LC/MS assay and ELISA

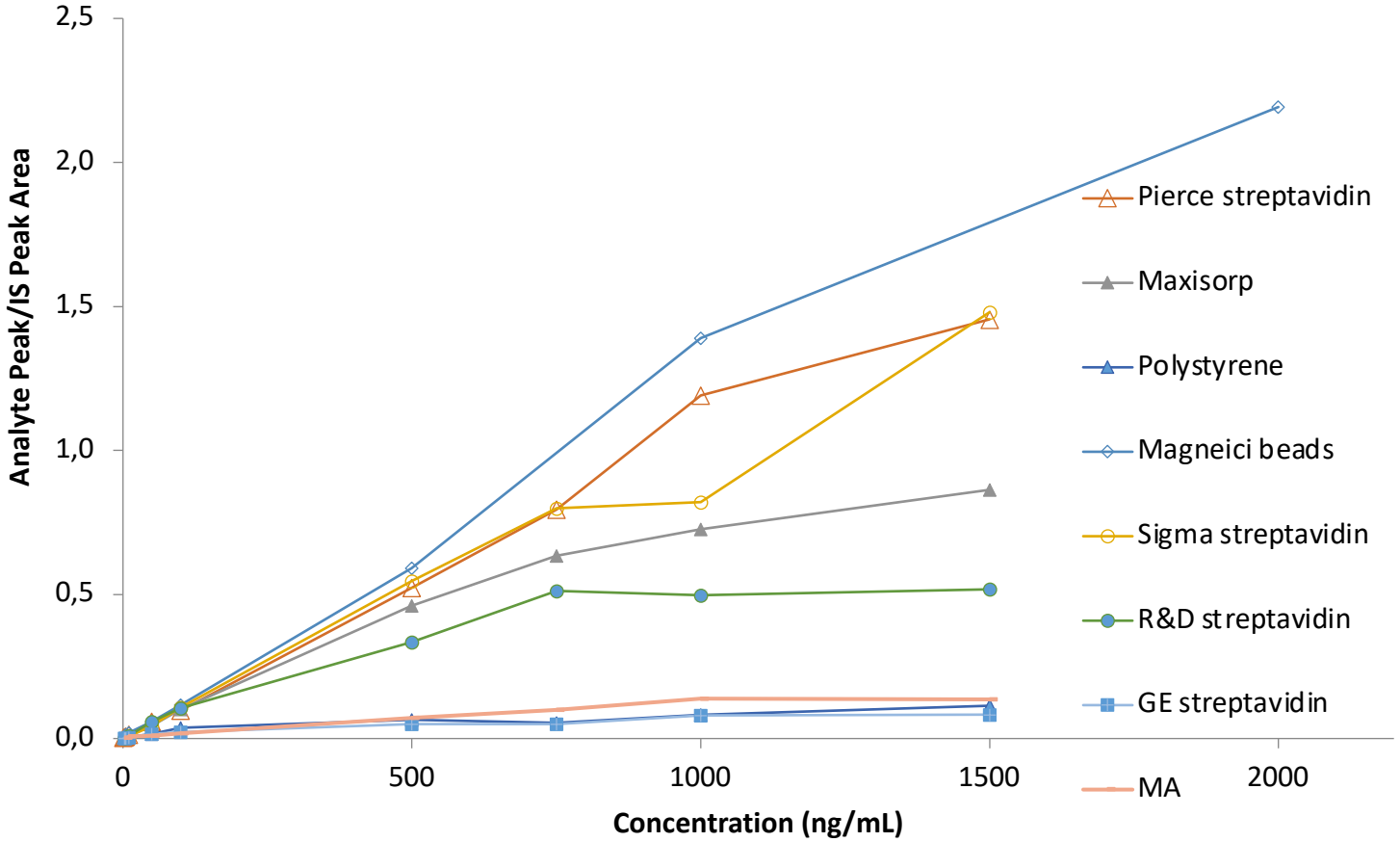
Hybrid Assay Workflow



LC/MS/MS System

- LC system
  - Waters Acquity UPLC
  - Column: Acquity UPLC BEH300 C18, 1.7µM, 2.1 x 50mm, 45°C
    - Mobile Phase: A, 0.1% FA; B, 0.1% FA in MeCN
    - Gradient: 0.3 ml/min, 5 min gradient
- MS/MS system
  - Sciex API 6500Qtrap:
    - +ESI, 5500V, 375 °C; gas 1/2: 50/50; Q1/Q3: unit resolution
  - Quantitation peptide MRM
    - EGVSAIR: 366.2 (2+) → 446.2 (y4)
    - IS: EGVSAI[13C6,15N4-R] 371.2→456.2

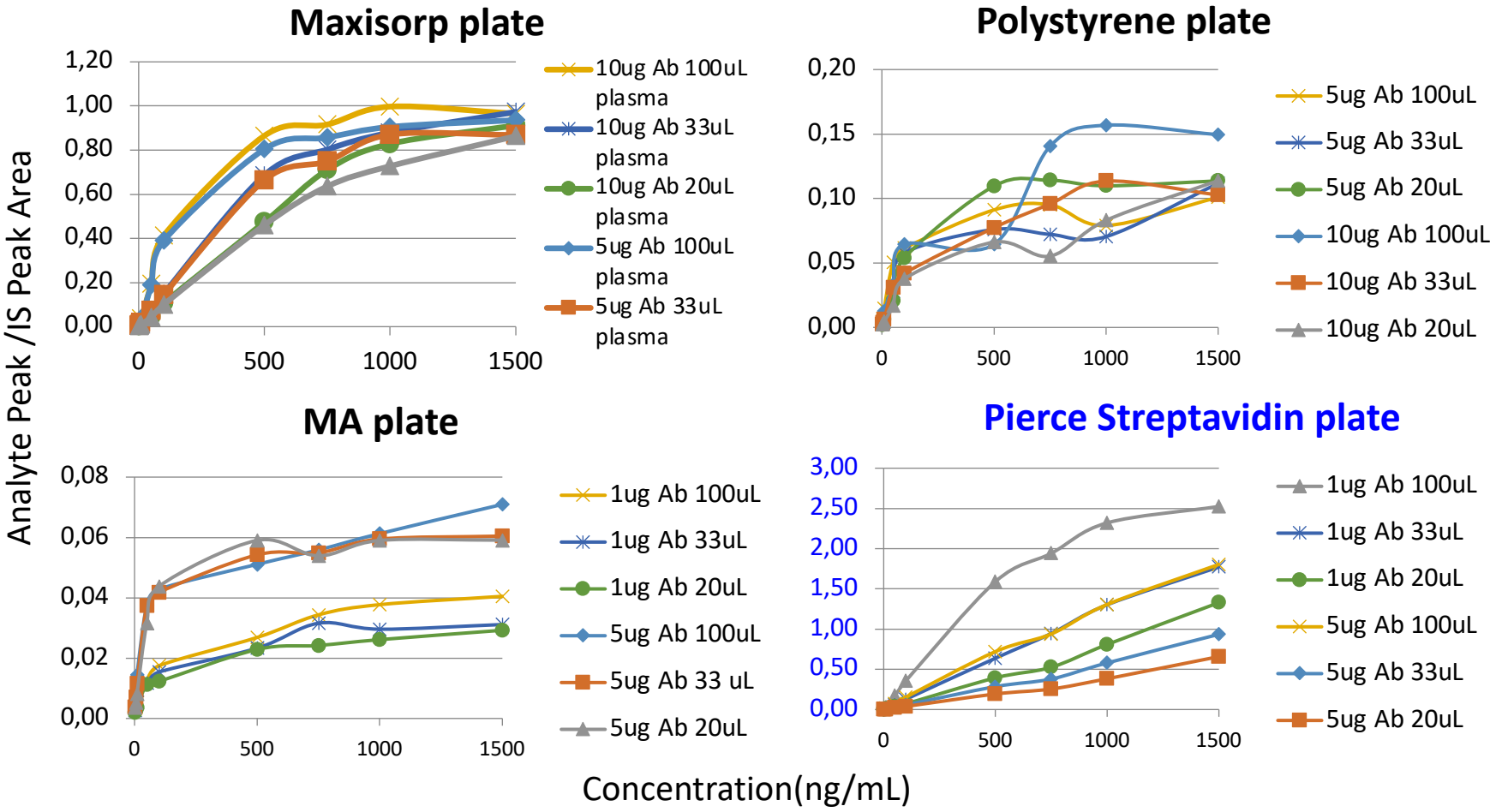
Hybrid assay LC/MS response
5 µg capture Ab



Pierce streptavidin plate has highest capacity and dynamic range, comparable to magnetic beads assay

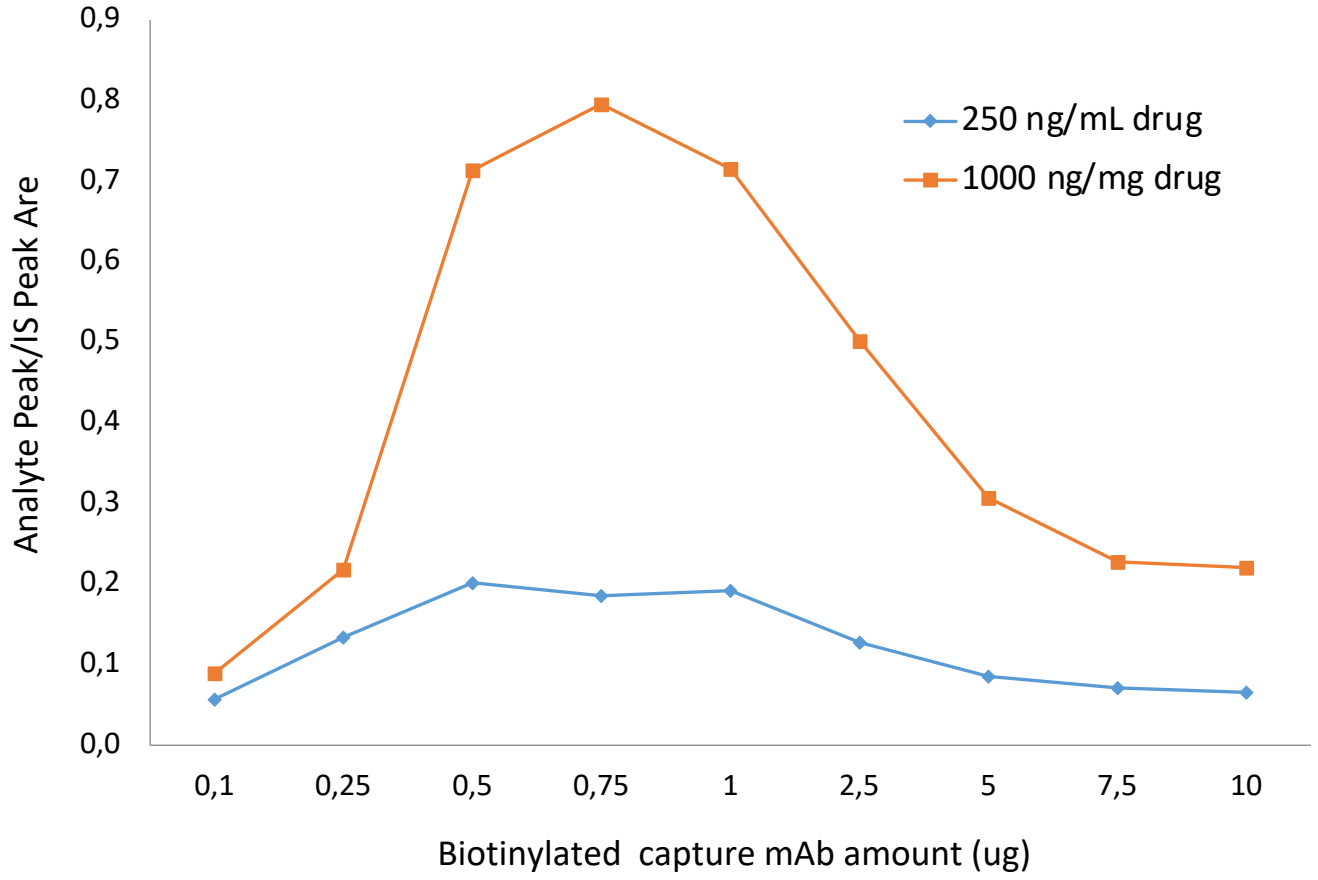
Dynamic range tests using different ELISA plates

LC/MS response of EGVSAIR

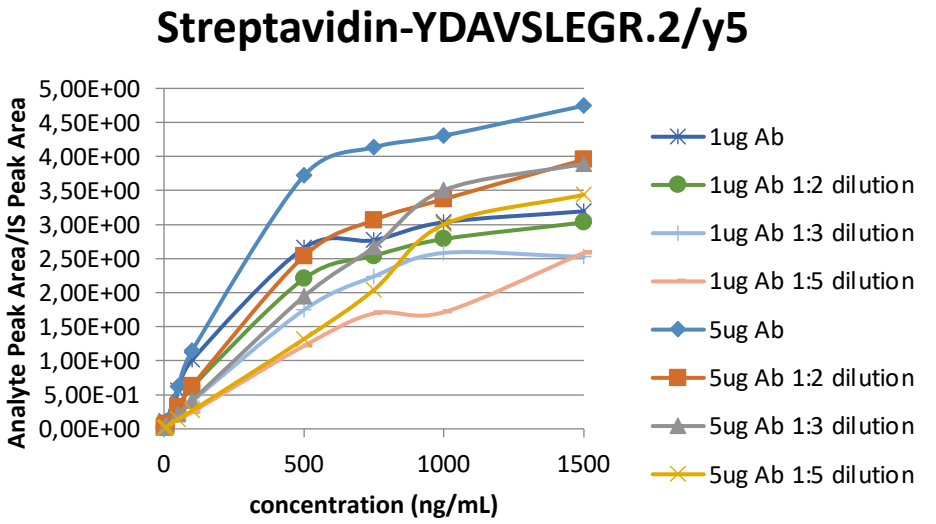


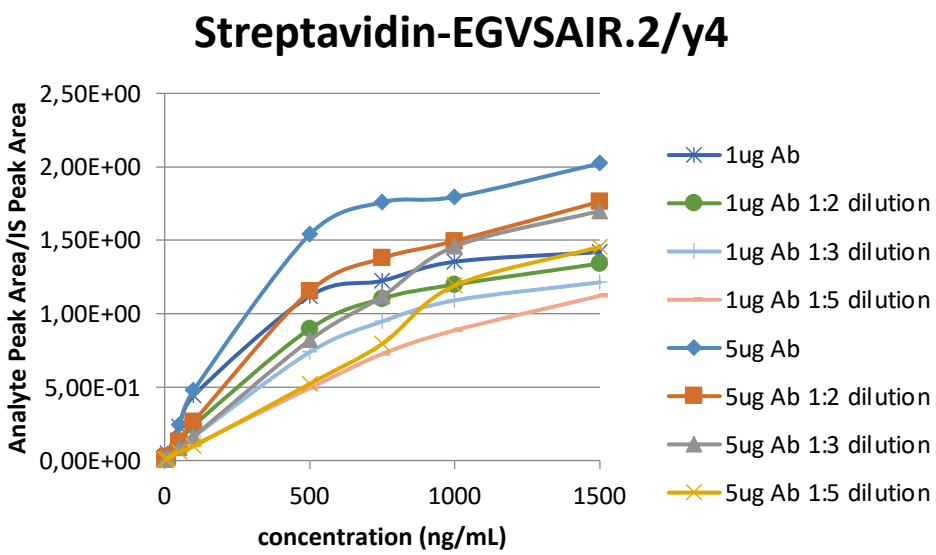
Pierce Streptavidin coated high capacity plate

LC/MS response *vs* antibody amount



Pre-blocked Pierce streptavidin plate





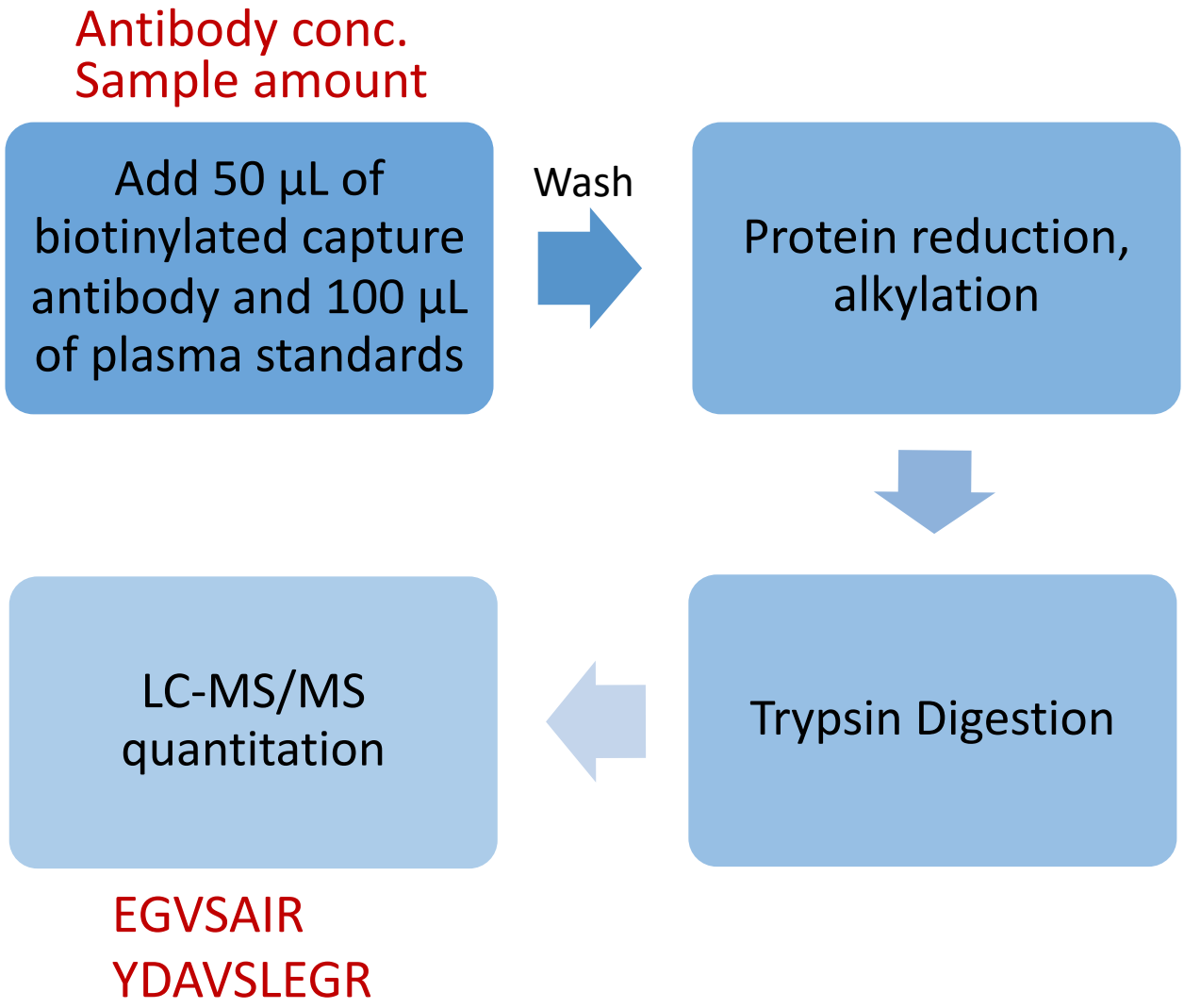
100µL plasma (no dilution)
1 µg Ab: 2.5-100 ng/mL
5 µg Ab: 2.5-100 ng/mL

50µL plasma (+50µL buffer; 1:2 dilution)
1 µg Ab: 2.5-500 ng/mL
5 µg Ab: 5-500 ng/mL

33µL plasma (+66µL buffer; 1:3 dilution)
1 µg Ab: 5-750 ng/mL
5 µg Ab: 5-1000 ng/mL

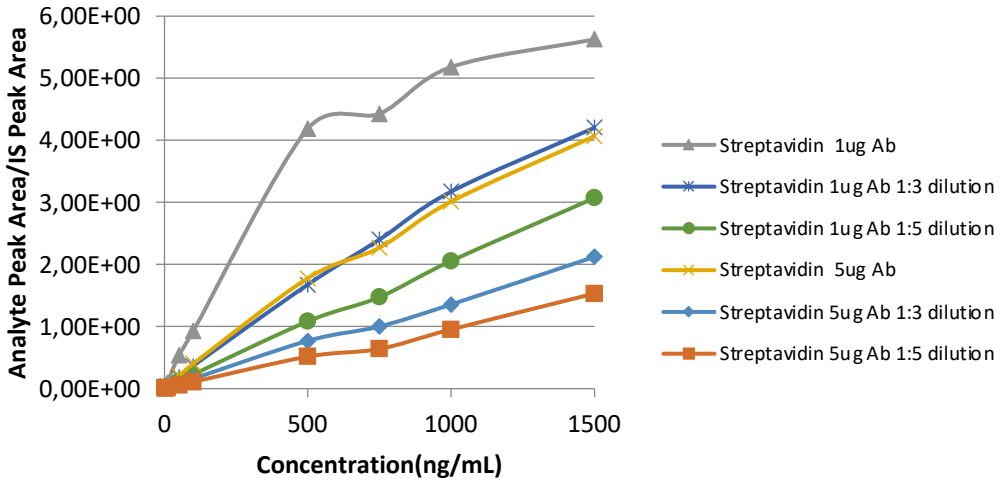
20µL plasma (+80µL buffer; 1:5 dilution)
1 µg Ab: 10-1000 ng/mL
5 µg Ab: 10-1500 ng/mL

Modified Streptavidin Plate Hybrid Assay



Non pre-blocked Pierce streptavidin plate

Streptavidin upadte-YDAVSLEGR.2/y5

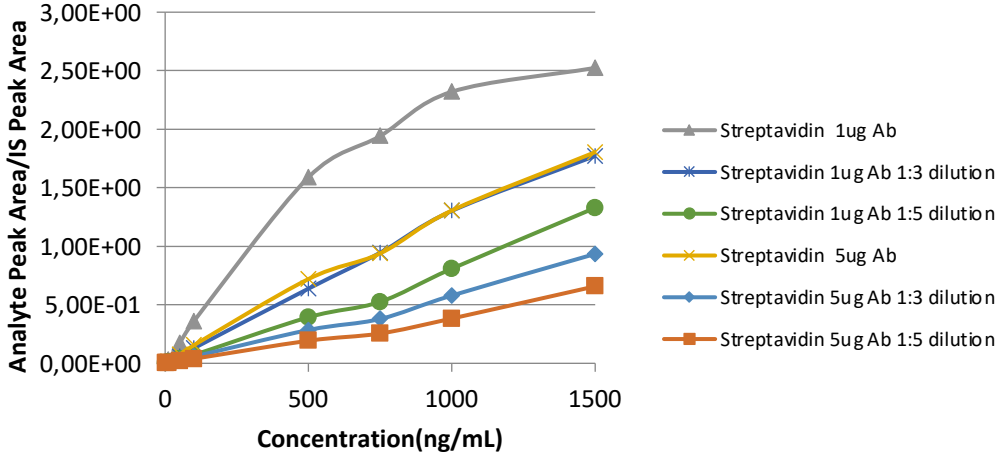


100µL plasma (no dilution)

1 µg Ab: 2.5-500 ng/mL

5 µg Ab: 5-1500 ng/mL

Streptavidin upadte-EGVSAIR.2/y4



33µL plasma (+66µL buffer; 1:3 dilution)

1 µg Ab: 5-1500 ng/mL

5 µg Ab: 10-1500 ng/mL

20µL plasma (+80µL buffer; 1:5 dilution)

1 µg Ab: 5-1500 ng/mL

5 µg Ab: 10-1500 ng/mL

Hybrid Assay Calibration Standard Performance

Pierce streptavidin coated plate with 1 µg Ab

EGVSAIR.2/y4	Concentration(ng/mL)								
	5	**10**	**50**	**100**	**500**	**1000**	**1500**	**2000**	**2500**
RUN1	5.07	9.57	51.6	108	498	1120	1450	1810	2350
	5.17	9.25	50.7	102	543	1020	1540	1960	2230
RUN2	5.18	9.20	47.0	118	517	1110	1450	1840	2230
	5.05	9.72	51.3	102	533	1060	1530	1940	2140
AVERAGE	5.12	9.44	50.2	108	523	1080	1490	1890	2240
RE%	2.3	-5.7	0.3	7.5	4.6	7.8	-0.5	-5.6	-10.5
CV%	1.3	2.7	4.3	7.0	3.8	4.3	3.3	3.9	3.9

Intra-Day QC performance

Pierce streptavidin coated plate with 1 µg Ab

EGVSAIR.2/y4	Concentration(ng/mL)				EGVSAIR.2/y4	Concentration(ng/mL)			
	LLOQ 5	LQC 15	MQC 750	HQC 1875		LLOQ 5	LQC 15	MQC 750	HQC 1875
DAY 1	6.02	14.3	906	1520	DAY 2	5.00	18.6	772	1650
	6.23	15.1	835	1930		4.97	16.9	828	1780
	5.88	14.0	842	1910		4.23	15.8	757	1840
	6.16	17.8	928	1790		4.42	18.4	897	1990
	6.08	13.7	894	1580		5.61	17.2	766	1640
	6.13	15.8	819	1950		5.53	15.9	784	1760
	6.20	14.4	832	1980		5.15	16.8	728	1800
	6.23	16.8	938	1760		5.95	18.0	888	1920
AVERAGE	6.12	15.2	874	1800	AVERAGE	5.11	17.2	803	1800
RE%	22.3	1.6	16.6	-3.9	RE%	2.2	14.7	7.0	-4.1
CV%	2.0	9.5	5.4	9.7	CV%	11.5	6.2	7.8	6.7

Inter-Day QC Performance

EGVSAIR.2/y4	Concentration(ng/mL)			
	LLOQ 5	**LQC 15**	**MQC 750**	**HQC 1875**
DAY 2	5.00	18.6	772	1650
	4.97	16.9	828	1780
	4.23	15.8	757	1840
	4.42	18.4	897	1990
	5.61	17.2	766	1640
	5.53	15.9	784	1760
	5.15	16.8	728	1800
	5.95	18.0	888	1920
DAY 1	6.02	14.3	906	1520
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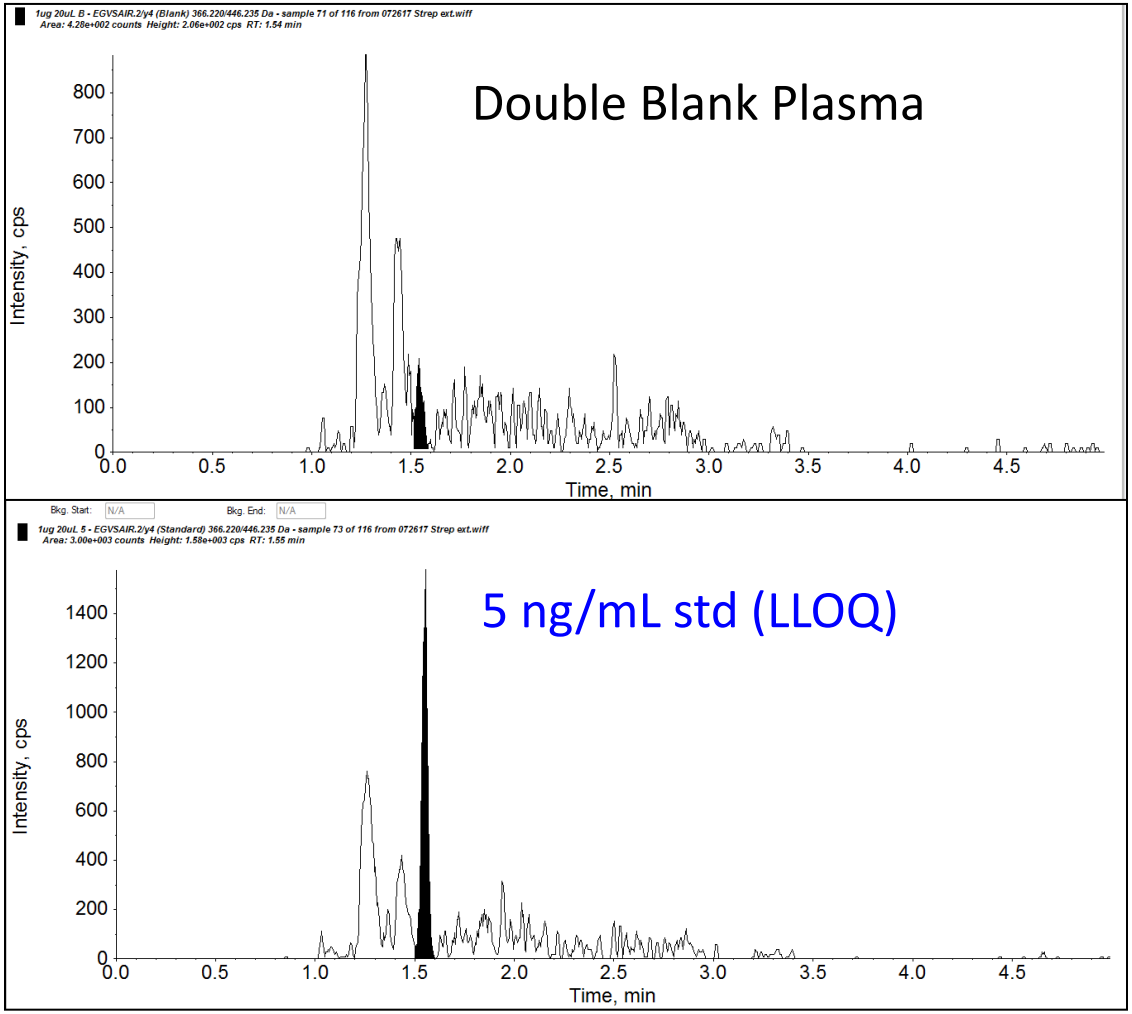
	6.08	13.7	894	1580
	6.13	15.8	819	1950
	6.20	14.4	832	1980
	6.23	16.8	938	1760
AVERAGE	5.60	16.2	838	1800
RE%	12.2	8.1	11.8	-4.0
CV%	11.8	9.8	7.8	8.0

Representative LC/MS Chromatograms

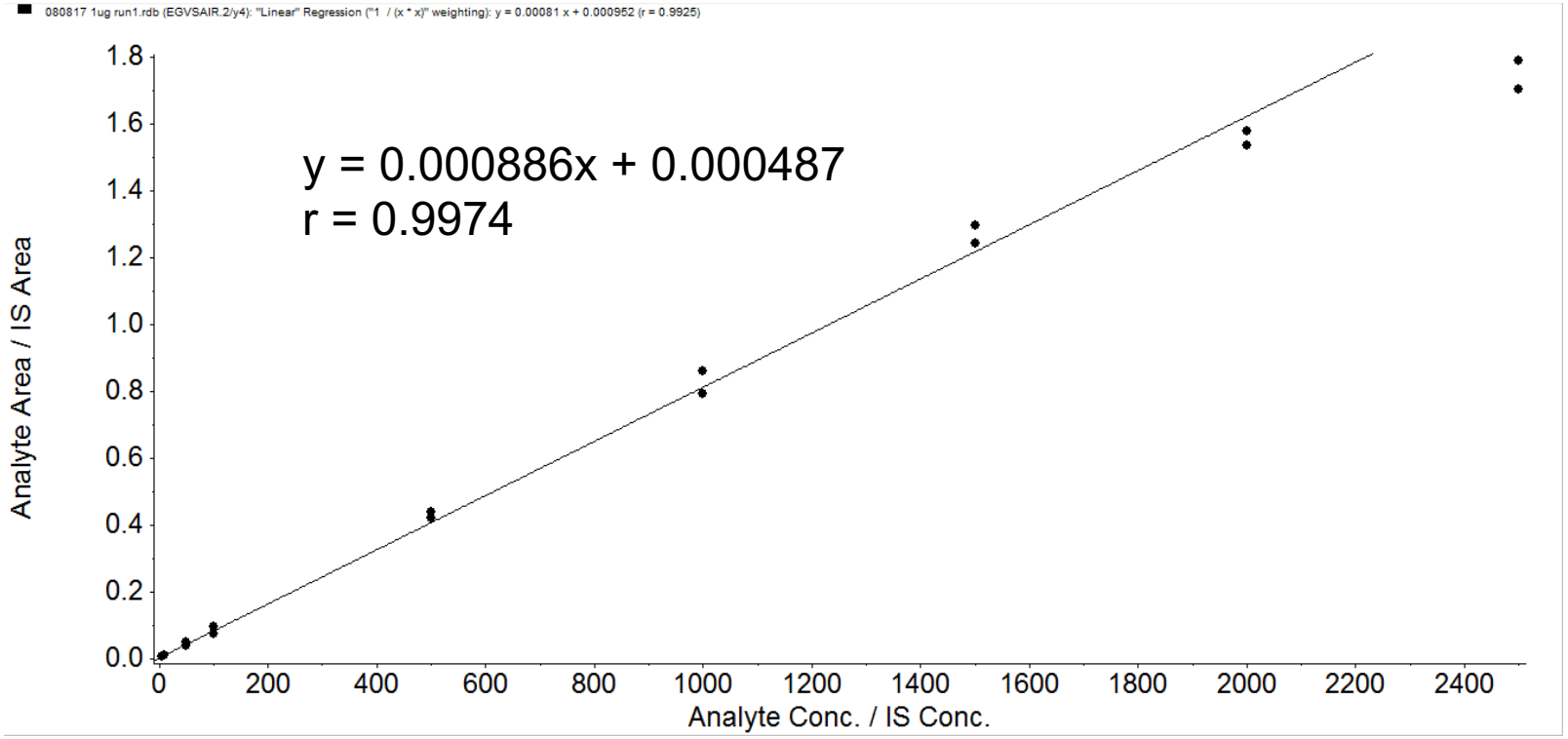
Pierce streptavidin coated plate



Representative LC/MS calibration curve

Pierce streptavidin coated plate with 1 µg Ab

5 – 2500 ng/mL



Hybrid *vs* beads *vs* ELISA

Parameters	Hybrid ELISA-LC/MS Assay		Magnetic beads-LC/MS		ELISA
Capture Ab	1 µg	5 µg	1 µg	5 µg	0.1 µg
Detection Ab	-	-	-	-	0.003 µg
Sample volume	20 µL	20 µL	20 µL	20 µL	20 µL (dil)
LLOQ	5 ng/mL	10 ng/mL	5 ng/mL	10 ng/mL	25 ng/mL
Assay range	5 – 2500 ng/mL	10 –5000 ng/mL	5 – 1000 ng/mL	10 – 5000 ng/mL	50 – 500 ng/mL
MRD	-	-	-	-	1:30.4

Summary

- 7 common ELISA plates tested
- Pierce streptavidin coated plate works the best
  - LOQ (5 ng/mL), dynamic range (5-2500 ng/m), similar the magnetic bead immunocapture-LC/MS assay and wider than ELISA (50–500 ng/ml)
- ELISA plate immunocapture provided a cleaner sample than the bead immunocapture, resulting in a lower LLOQ
- ELISA plate—LC/MS hybrid assay
  - Assay performance similar to beads assay
  - Could be easily automated using a liquid handler
  - Lower cost

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