NEXT GENERATION SAMPLE PREPARATION FOR MS ANALYSIS OF TARGETED PLASMA METABOLITES

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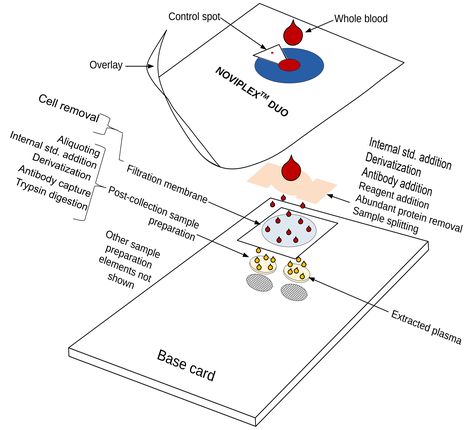
OVERVIEW AND INTRODUCTION

As a prelude to mass spectral (MS) analysis of blood samples blood must be drawn, the cellular components removed, a sample aliquot taken, internal standards are often added, analytes extracted, and multiple modes of fractionation applied before mass analysis can be achieved. These steps are either achieved manually or by parallel processing; often in multiple laboratories. The complexity of this process presents a series of problems ranging from poor reproducibility and difficulties in sample tracking, to how samples are transported, the need for trained personnel, and cost. This presentation will describe an automated, miniaturized system that achieves multiple sample preparation steps by capillary action or diffusion within membranes that circumvents many of these problems.



Schematic of the Noviplex™ Duo plasma extractor.

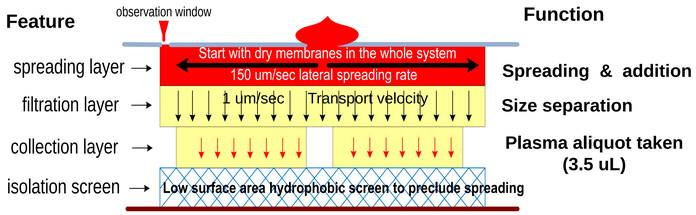
Objectives

1. Collect an aliquot of plasma, independent of the application volume.
2. Aliquot is hematocrit independent by removing cells before plasma collection.
3. Reagents and internal standards can be incorporated into samples.

Reference:

Anal. Chem., **2013**, *85* (23), pp 11501–11508.

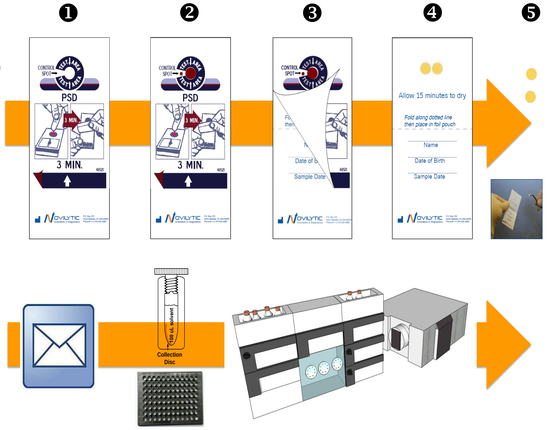
Design feature for dual plasma extraction (Duo)



NOVIPLEX™ PLASMA CARD EXTRACTION TECHNOLOGY

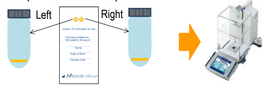
<Method and Results> Noviplex™ Duo plasma card Workflow

1. Remove Noviplex™ card from packaging.
2. An unmeasured application of about 60 µL of whole blood (one to two drops) is added to the test area.
3. After 3 minutes, the top layer is completely removed (peeled back).
4. The collection disc contains ~3.5 µL of plasma.
5. The collected sample is air-dried for 15 minutes.
6. After removal of the two identical collection discs from the base card analytes are ready extracted for LC-MS/MS analysis.
7. An appropriate concentration of an Internal standard may be added before extraction.



Validation of liquid collection volume and hematocrit variability

- 60 µL of fresh venous blood (EDTA; hematocrit 43%) was applied to each card.
- After 3 minutes, the card overlay was removed and discs were placed individually into left and right labeled vials.
- Loaded mass was determined for each vial using Mettler H54AR that is traceable to NIST standards.
- Measured volume is calculated into volume by using a specific gravity of plasma (1.0205 at 37°C).



Average collection volume from left and right disc is 3.52uL (±3%). Moreover, the variation between left and right was 2.8%.

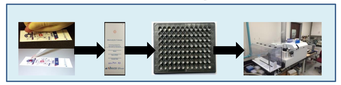
Hematocrit effect:

	Blood1	Blood2	Blood3
Hematocrit level	20%	43%	70%
Average collection volume (µL)	3.2	3.54	3.31
%CV	4.37%	2.62%	3.30%

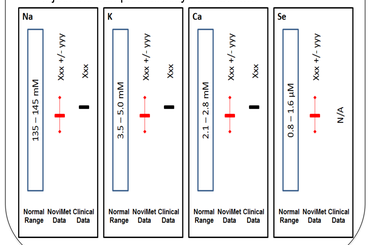
Variation of the collection volume among different hematocrit levels was 3.35uL ± 5.2%.

Ion measurement

Determination of Na, K, Ca and Se levels in plasma

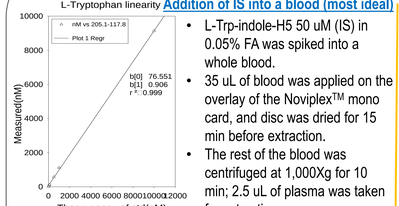


- Process blood into Noviplex™ card and transfer collection disk into a well of the 96-well plate.
- Add 10 µL nitric acid (100%) and incubate for 2 hrs at RT and add 190 µL water and incubate for 1 hr at RT.
- Add 200 µL Internal Standard (100 ppb Ga in water)
- Inject the samples directly into ICP-MS



METABOLITES MEASUREMENT WITH NOVIPLEX™ CARD

Amino acid measurement and addition of IS

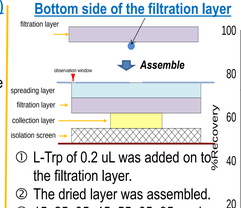


Addition of IS into a blood (most ideal)

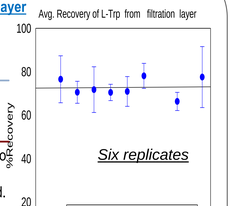
- L-Trp-indole-H5 50 uM (IS) in 0.05% FA was spiked into a whole blood.
- 35 uL of blood was applied on the overlay of the Noviplex™ mono card, and disc was dried for 15 min before extraction.
- The rest of the blood was centrifuged at 1,000Xg for 10 min; 2.5 uL of plasma was taken for extraction.

Noviplex (A), Avg. (uM)	Centrifugation (B), Avg. (uM)	A/B	%RSD (A vs B)
69.83	72.00	97%	2.16

Bottom side of the filtration layer

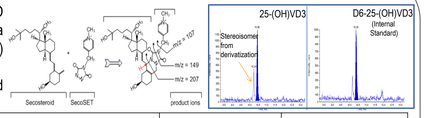


① L-Trp of 0.2 uL was added on top of the filtration layer.
② The dried layer was assembled.
③ 15, 25, 35, 45, 55, 65, 85, and 100 uL of blood were applied to collect plasma samples.
④ Heavy isotope IS was added and extracted.



Vitamin D measurement

Noviplex™ Duo plasma cards were used in the analysis of vitamin D blood levels from a cohort of human blood and NIST SRM 972a standards. The SecoSET vitamin D derivatization kit (Novilytic, IN) was used to enhance vitamin D signals in MS.
Shimadzu HPLC system (Shimadzu, Japan) coupled to the Applied Biosystems/MDS Sciex 4000 QTRAP was used for MRM method .



	from a disc (ng/mL)	NIST measured (ng/mL)	%Accuracy
NIST SRM 972A (plasma)	13.7	12.4	92.96%
Reproducibility (%CV)	1.8	-	

	Noviplex Duo (left)	Noviplex Duo (right)
Donor A (ng/mL)	28.99	27.53
plasma volume on disc (uL)	3.5	3.5
Reproducibility (%CV, n=6)	11.9	11.35

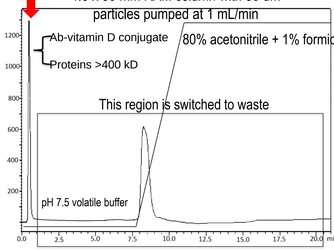
%Error was 3.65% using 6 replicates.

Nanoparticulate affinity sorbents (NAS)

MSAC (mobile sorbent affinity chromatography) was created to isolate substances of interest exclusively.

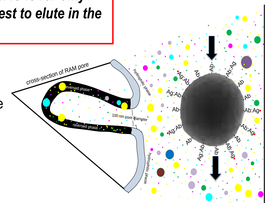
Immune complex(Ab:Ag)

4.6 x 50 mm RAM column with 50 um particles pumped at 1 mL/min



The objective in MSAC is for only substances of interest to elute in the solvent front.

Metabolites and most proteins enter the RAM pores but not the nanoparticles.



Retention time of the Ab:Ag particle is 30 sec at 1 mL/min. Peak width <15 sec. Preliminary data shows that multiple samples can be run without recycling.