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# Untargeted metabolomics on dried blood spots

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## Introduction

Dried blood spot (DBS) samples were initially used in hospitals in newborns for screening congenital diseases. Dried blood spot analysis is a very promising alternative to conventional whole blood and plasma analysis used in clinical studies in particular due to its advantages such as the non-invasive collection method, the low sample volume required and the ease of sampling which can be performed at home without the intervention of medical staff. To date, there is little work on dried blood spots in untargeted metabolomics approaches because of inhomogeneous samples due to differences in the volume of the blood samples spotted on the filter paper and fluctuations in haematocrit from one sample to another. Normalization strategies must be performed (haematocrit quantification, fixed volume microsampling devices) in order to be able to compare metabolic profiles between them.

The first objectives of this work are: to study the effect of the extraction solvent and to study the effect of the micro-sampling support. The first analyses are carried out on human serum samples spotted on filter paper to observe the impact of the support on known samples before studying whole blood samples not analysed within the PFEM until now.

Objective 1: Extraction solvent comparison

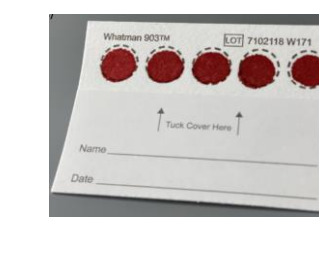


MeOH vs Water/MeOH 20/80

Objective 2: Effects of different sampling techniques



Mitras volumetric absorptive microsampling device (VAMS) by Neoteryx®

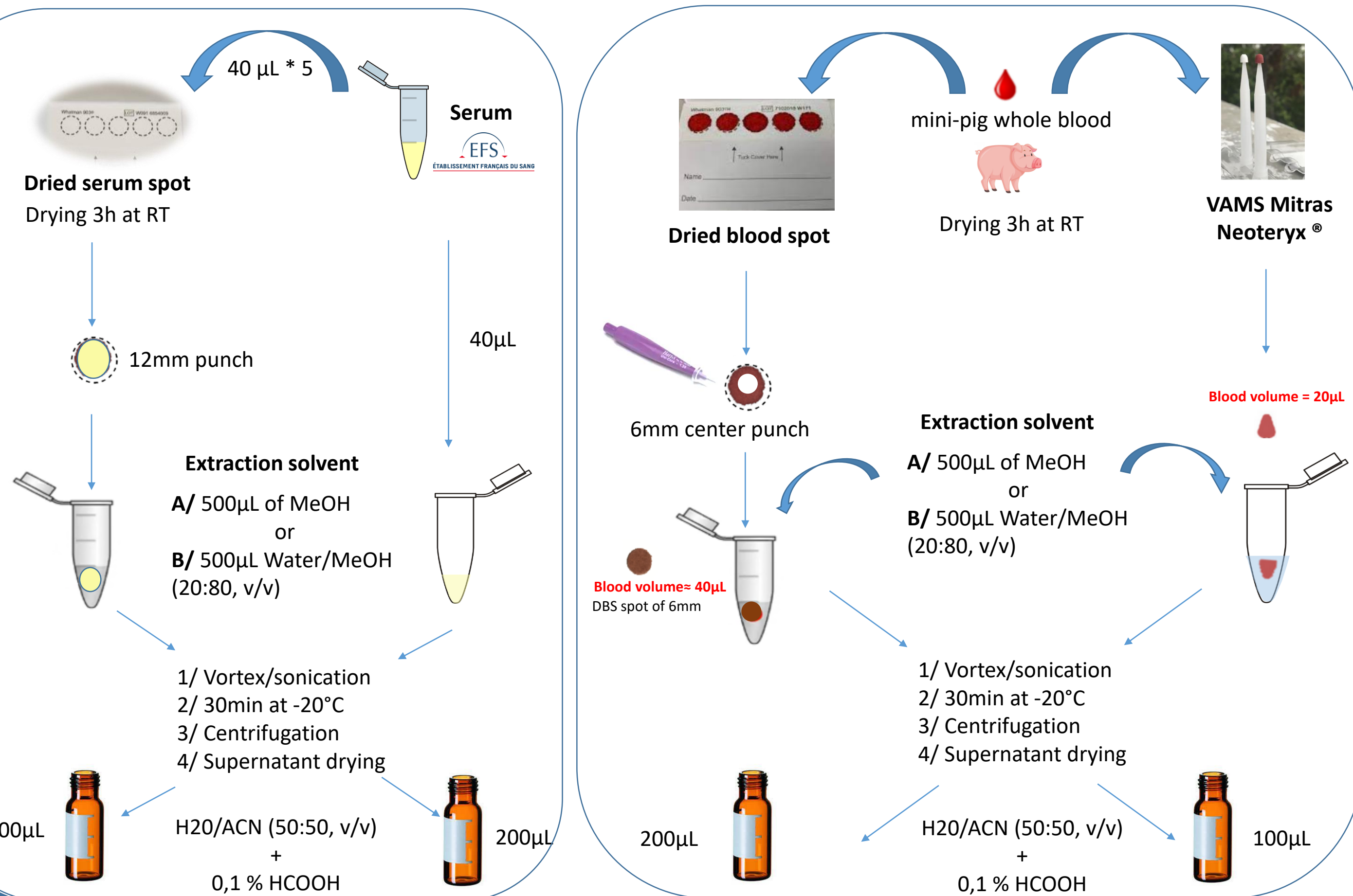


Whatman 903 protein saver: Standard dried blood spot card

## Materials and Methods

1 / Experiment on human serum samples

2 / Experiment on whole blood samples



LC-MS analysis

- LC conditions: UHPLC U3000 (Thermo)
- 150 x 2.1mm Acquity HSS T3 C18 column (Waters)
- Flow rate : 0.4mL/min
- Mobile phase: 100% water (A) and 100% acetonitrile (B) both containing 0.1% formic acid

Retention [min]	Flow [mL/min]	SA	SB	Curve
1	0.000	0.400	100.0	0.0
2	0.000	0.400	100.0	0.0
3	2.000	0.400	100.0	0.0
4	15.000	0.400	0.0	100.0
5	22.000	0.400	0.0	100.0
6	22.100	0.400	100.0	0.0
7	26.000	0.400	100.0	0.0

- Column temperature: 30°C
- Injection volume : 5 µL
- Mass conditions: TimsTOF (Bruker)
- Ion modes: ESI+/ESI-
- Scan range: 50 to 1000m/z, Full scan

Parameters	Positive ion mode
Extraction Method	CentWave
ppm	10
PeakWidth	5.20
Smooth	10
PreFilter	3.1000
msCentrifun	Use the m/z value at the peak apex
Modif	0.01
Method	PeakDensity
bw	8
minFraction	0.9
minSamples	2
binSize	0.01
maxFeatures	100
Method	PeakGroups
minFraction	0.9
extraParams	1
smooth	1000
aper	0.2
family	Gaussian
the	1
Convert rt (sec) into min	yes
Decimal places mass values	4
Decimal places rt values	2
initial	maxo

Untargeted processing approach

Parameter	Value
sigma	6
perfusion	0.6
ppm	5
maxCharge	3
maxion	4
minfrac	0.5
Max_peaks	100
Number of condition	One condition

Comparison by sample class  
Sample blank filtered data

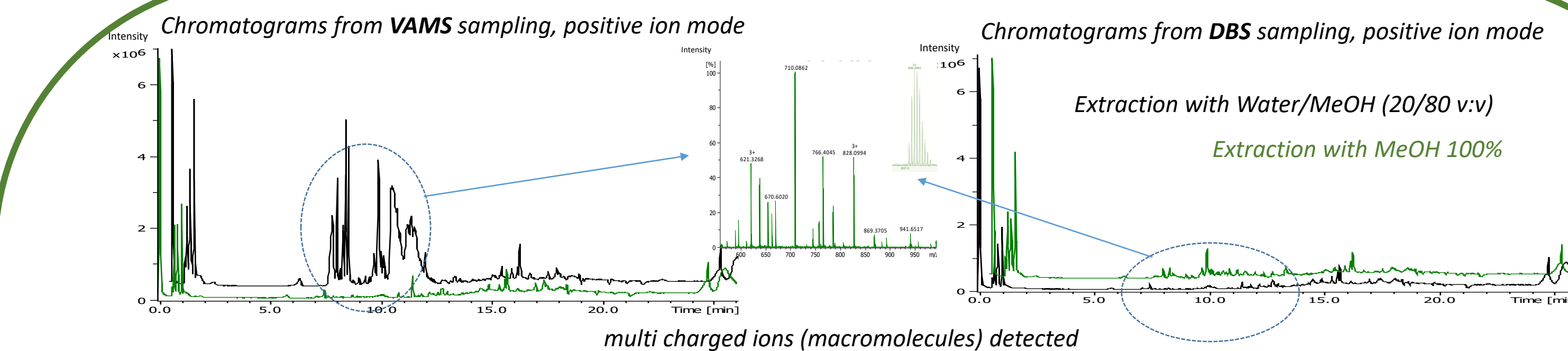
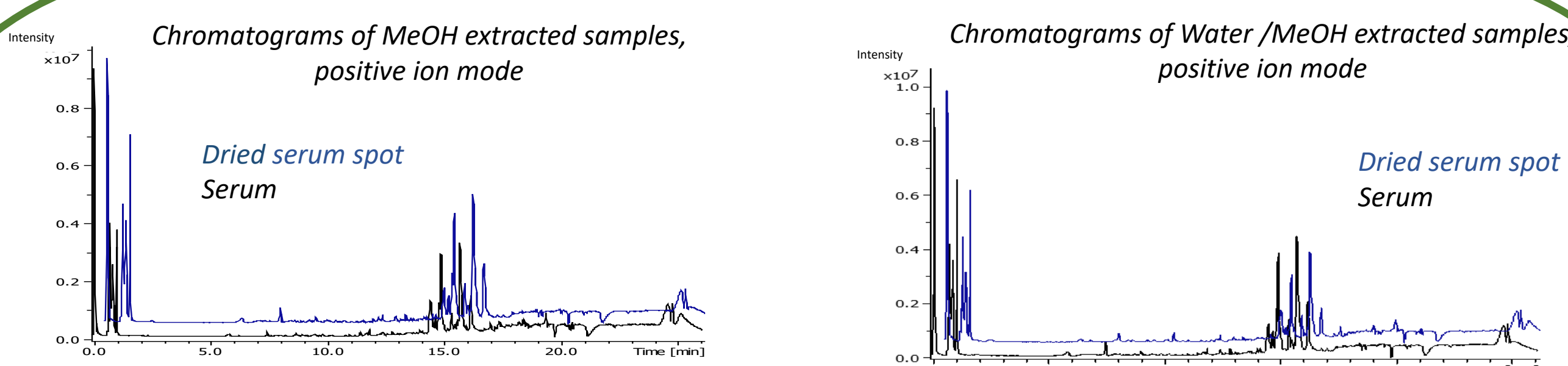
Targeted processing approach



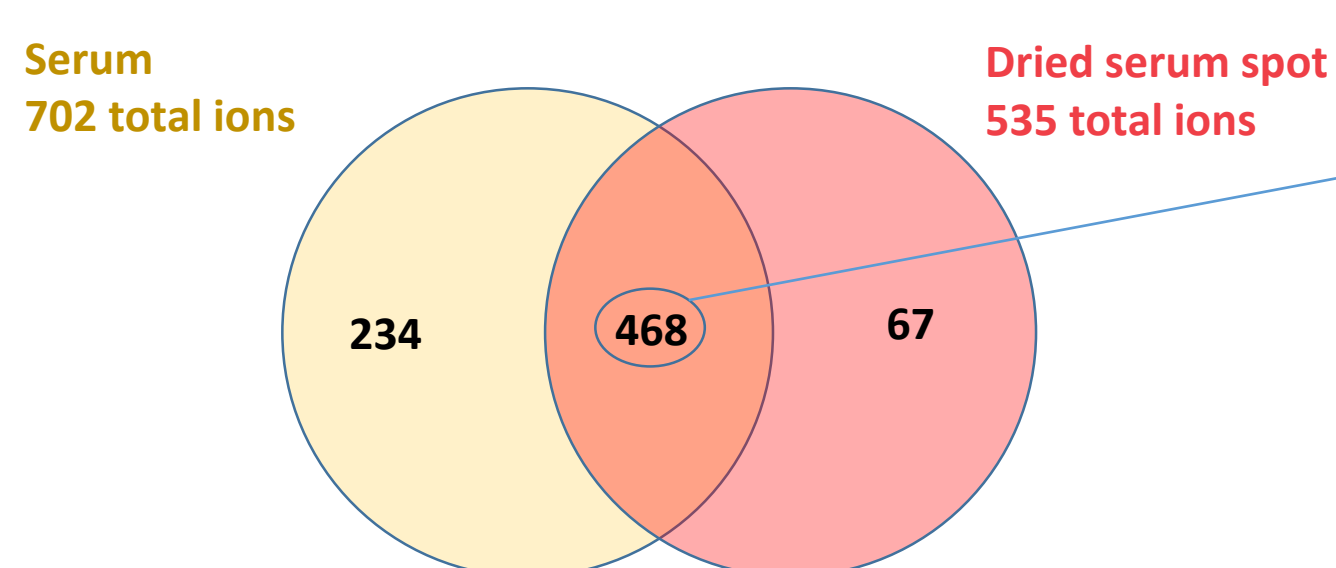
SERUM SAMPLES

## Results

WHOLE BLOOD SAMPLES



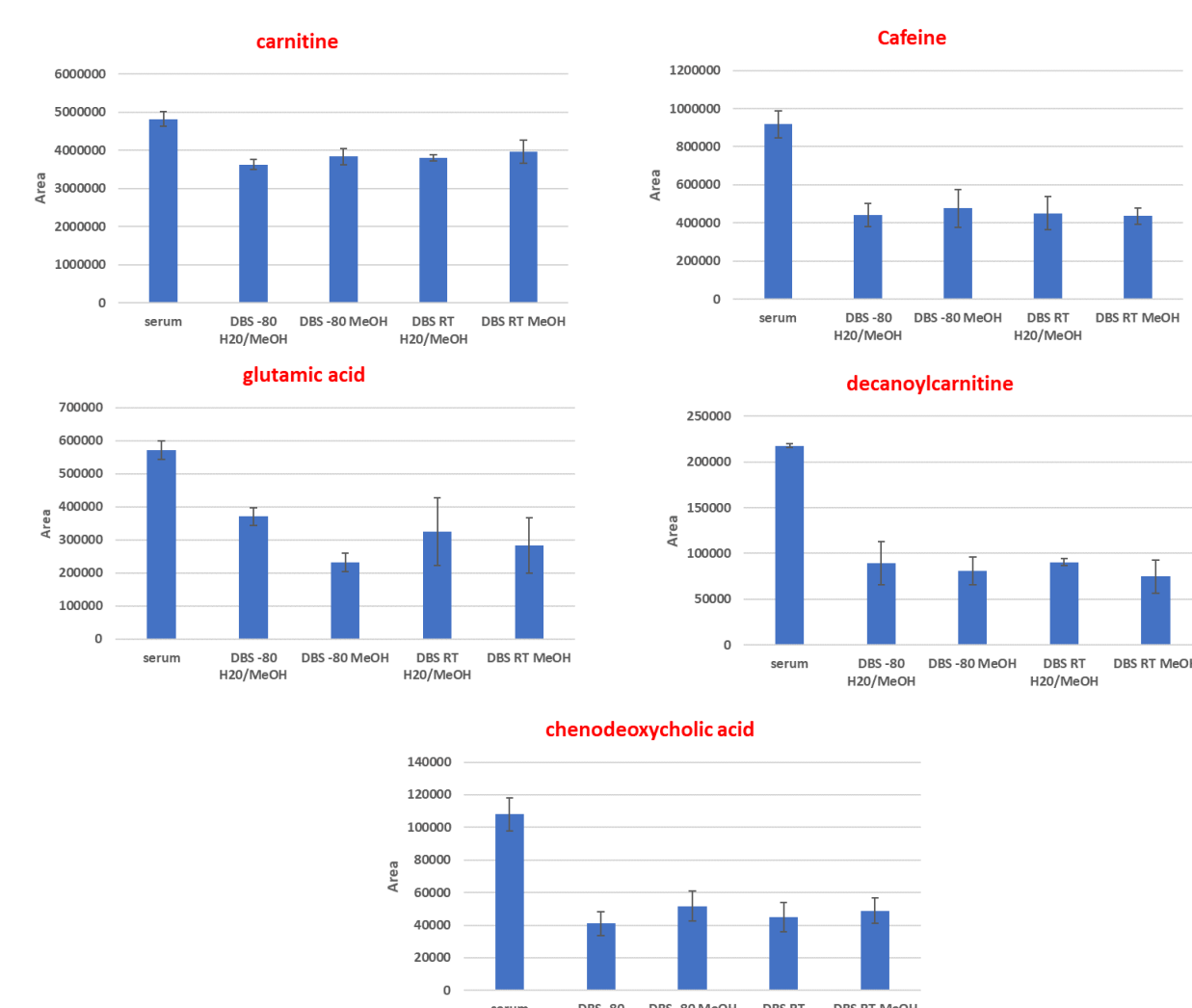
Untargeted processing approach



- 11% of annotated features with our databases: Amino acids and derived, carnitines and derived, dipeptides, phospholipids, carbohydrates.
- 87% of the ions detected on the DBS are common to the ions detected in the serum.
- Some metabolites are specifically observed in dried serum samples.

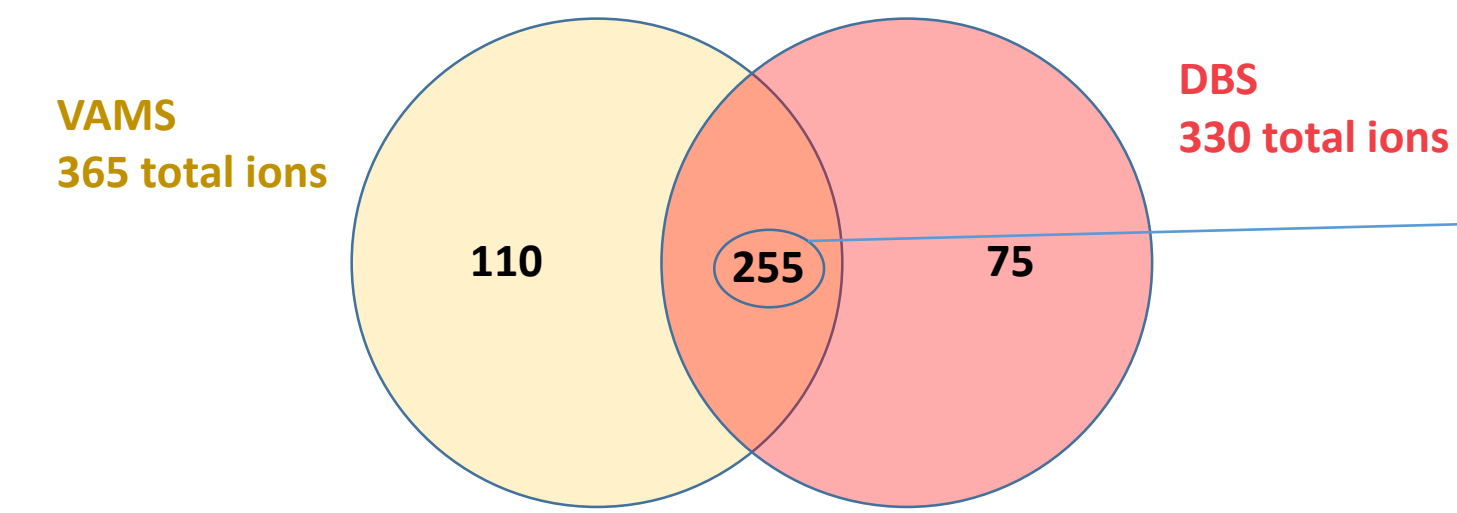
Comparison of the extracted ions between the 2 modalities, serum and dried serum spot (Extraction A, 100% MeOH)

Targeted processing approach



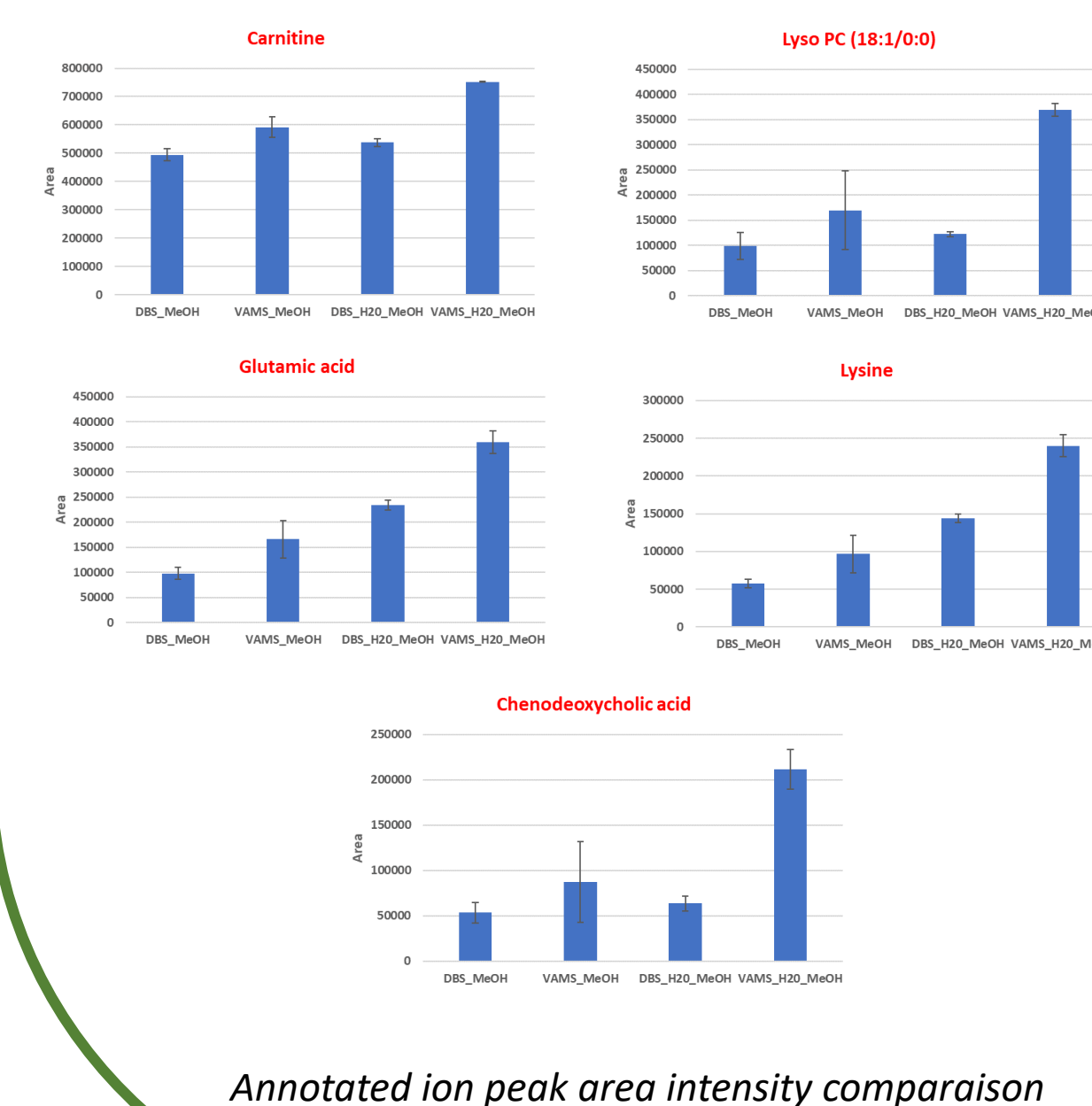
- A higher percentage of high mass ions ( $m/z > 500$ ) is measured in dried serum samples (43%)
- The ion  $m/z$  distribution is similar regardless of the extraction solvent (results not shown)
- The extraction yield depends on the metabolites. Carnitine is the best extracted among the target metabolites.
- Freezing effect on the DBS cards was tested (RT vs -80°C): no significant difference was observed.
- Similar results between the 2 extraction solvents, confirmed by the untargeted processing approach (results not shown)

Untargeted processing approach



Comparison of the extracted ions between the 2 modalities, VAMS and DBS (Extraction A, 100% MeOH)

Targeted processing approach



- 11% of annotated features with our databases: Amino acids and derived, carnitines and derived, dipeptides, phospholipids, carbohydrates.
- 3/4 of the ions detected are common to all supports tested.
- the number of extracted ions is similar for all devices tested.
- With methanol extraction few ions are measured compared to the number of ions observed in a serum sample.

- The percentages of high mass ions ( $m/z > 500$ ) measured are the same regardless of the devices (Extraction A, 100% MeOH)
- Many multi-charged ions are extracted with extraction protocol B (results not shown) => protein precipitation not effective?

- As part of the targeted approach all metabolites are extracted and detected regardless of the support tested.
- VAMS devices show better extraction on the few targeted metabolites regardless of the extraction solvent.
- For the same support tested, it seems that the water-methanol extraction solvent is the most efficient

## Conclusion and Perspectives

The experiment on human serum spotted on DBS cards shows that some metabolites are specifically measured in the dried serum samples => *Drying the sample for 3 hours at room temperature did it change the observed metabolic profile?* Our results shows that fewer metabolites are extracted in dried serum sample, which is consistent with results from the literature [1] => *Are the metabolites adsorbed on the DBS card?*

The dried blood spot is a promising novel method which required low blood volume for blood collection. This non-invasive sampling method has other advantages including feasibility of patient self-sampling, collection of blood easily performed at home and facility of storage. The water/methanol extraction solvent seems to provide better results. However, the presence of macromolecules in the extracted samples could induce bias.

The analysis of whole blood samples will open new opportunities for exploring intracellular metabolites present in red blood cells. This matrix provides better coverage of the metabolome compared to the plasma or serum samples [2].

Next steps: Continue processing data acquired with VAMS & DBS sampling, continue storage stability studies at different temperatures, continue long-term storage stability studies...

References : [1] Kong et al. 2011 *Analytical chemistry*, 83 (11) : 4314-4318; [2] Volani et al. 2023 *Metabolomics*, 13 : 146

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Plate-Forme EXPLORATION du MÉTABOLISME des gènes aux métabolites

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