

NMR as a general tool for assessing plasma sample quality

Anders Pedersen

Nordic Biobank Conference 2022











Preanalytical 'aging' of plasma samples: pilot study (unpublished)

September 6-8, 2022 • #nordicbiobank2022 • nbc.biobanksverige.se

NMR spectroscopy

- Detects 'NMR active' nuclei, e.g. ¹H, ¹³C, ¹⁵N, ³¹P
- Inherently quantitative
- Fully automatable
- Fantastic reproducibility
- Typical biofluid sample a complex mixture of metabolites in a wide concentration range, always with spectral overlap







Samples fit-for-purpose: metabolomics

What happens *before* centrifugation?

Time scale for metabolomic changes in the seconds to minutes range

Pilot case: EDTA plasma tubes with gel separator; 27 participants, 2 occasions, a total of 951 samples with acquired data for analysis

Parameters varied:

- Temperature (4°C, 25°C, 37°C)
- Time (5 min 210 min)
- Ambient light status (yes/no)

Multivariate analysis of ¹H NMR data: 325 peaks







Analysis with persons' samples as groups



Predict sample preprocessing 'age'

E Biobank

Approximate measured concentrations of my sample to 'mean aging' value of the model instead of the actual aging.

What happened to my sample prior to NMR analysis? Assume same as nearest neighbours.

Can I use the model on my sample? Must be similar to the samples used in the model. Not too different within (Hotelling's T2) or much outside (Distance to) the model.

Sample aging (orthogonal score space)



Biobank

Conference

- Temperature and time show distinct effects
- Ambient light status no obvious effect



 4°C stable after initial change

Temperature

- 37°C fastest change
- Different temperature different pattern

September 6-8, 2022 • #nordicbiobank2022 • nbc.biobanksverige.se



SCFAs Pyruvate

Lactate, Ornithine, ...

4°C

a Biobank Sconference



Short-chain fatty acids (SCFAs)



- Effect at 4°C prompted follow-up experiment
- Not unspecific: buffer + SCFA mix in EDTA tubes does not show this effect
- Pre-centrifugation cold temp gives *apparent* lower SCFA concentrations
- **Reversible** effect suggests that plasma should be handled at room temperature ASAP or equilibrated to room temperature before centrifugation



September 6-8, 2022 • #nordicbiobank2022 • nbc.biobanksverige.se

Ambient light status

E Biobank

- Subtle effect but robust model
- Effect independent of time and temperature
- Of less practical importance. Time and temperature more important
- 'Light' samples have broader lines effect of release of *e.g.* paramagnetic Fe^{2+}/Fe^{3+} ?



<mark>Light-Dark</mark> Dark-Light



2-oxoisocaproate 3-hydroxybutyrate



Tube type assessment and Biobank QC with NMR (in use and under development)

September 6-8, 2022 • #nordicbiobank2022 • nbc.biobanksverige.se

Impact of sampling tube type



EDTA tubes with gel separator contributes 'metabolites':

- acetate
- formate
- N,N-dimethylglycine
- sarcosine
- propylene glycol

Choice of sampling tube matters!!



Sampling tube type

Apart from serum glass tubes vs. LiHeparin w/o gel separator, **all** tested tubes can be identified spectroscopically





[ppm]

• Requires adherence to strict sample preparation, spectrometer maintenance and data acquisition SOPs, *i.e.* the Bruker IVDr protocols.

- Worldwide exchangeability of NMR data between IVDr sites/biobanks
- Delivers information on pre-analytics, sample preparation and analytical performance.
- For serum, plasma, CSF and urine. Fully automated procedure.
- Ongoing development new features released continuously

CSF and urine



September 6-8, 2022 • #nordicbiobank2022 • nbc.biobanksverige.se

Biobank quality control



Test	Result	Flag
NMR Experiment Parameter Test	passed	
NMR Experiment Quality Test	passed	
NMR Preparation Quality Test	passed	
Matrix Identity Test	Urine	
Matrix Integrity Test	passed	
Matrix Contamination Test	passed	
Medication Test	not passed	•
Protein Background Test	passed	$ \bigcirc$
Further Indicative Parameter Test	not passed	

4 Test for Medication, Protein Background and further indicative Parameters

4.1 Medication and related Metabolites

Compound	LOD	Measured Value	Flag
D-Mannitol in mmol/L	0.150	<0.150	
D-Mannose-alpha in mmol/L	0.060	0.091	
Paracetamol in mmol/L	0.060	< 0.060	
Paracetamol-glucuronide in mmol/L	0.060	0.771	•
Paracetamol-sulfate in mmol/L	0.060	0.784	
Cefuroxim in mmol/L	0.500	<0.500	





Use ¹H NMR for spot-checks of biobank collections to assess overall sample quality?

Acknowledgements

Swedish NMR Centre Daniel Malmodin Göran Karlsson Millie Rådjursöga

Sahlgrenska Academy & Biobank Väst Åsa Torinsson Naluai Linda Paulson Huma Zafar

Bruker BioSpin Hartmut Schäfer

Roland Leiminger





SciLifeLab

Contact: anders.pedersen@nmr.gu.se

HASSEIBLADSLABOR

SwedNMR