

NMR Analysis of Human Serum Samples from the Alzheimer's Disease Neuroimaging Initiative Using the Nightingale Health Platform

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Objective

Quantification of routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular metabolites including amino acids, ketone bodies and gluconeogenesis-related metabolites from the ADNI 1/GO/2 cohorts' human serum samples using Nightingale Health's NMR metabolomics platform.

Introduction

Nightingale Health has developed a Nuclear Magnetic Resonance (NMR) based blood biomarker analysis assay, quantifying 250 metabolic biomarkers from a single blood sample. The result is a comprehensive molecular snapshot, covering biomarkers from multiple biological pathways and providing insights into human metabolism.

NMR and mass spectrometry have become key technologies in the metabolomics field. However, Nightingale's NMR platform has an advantage when compared to mass spectrometry approaches as there are no batch effects and it provides absolute concentrations of metabolic measures, rather than relative concentrations. Absolute concentrations increase the interpretability of the biomarker data provided.

Nightingale Health 2020 analysis update brings important changes that enable clinical use of Nightingale's biomarkers. The update improves consistency between different sample volumes and NMR instruments and yields even better match between biomarkers measured with clinical chemistry or other platforms. The updated results include CE-marked biomarkers that can be used for clinical diagnostics.

Sample Preparation

The samples were delivered in one batch. The batches were received on August 10th in good condition and placed into -80°C freezer

1. Samples were thawed overnight at +4°C (number of thawed samples was the same as number of samples what would be measured on the next day).



2. Sample preparation and measurement took place between September 1st and September 23rd.
3. Before the samples were prepared, they were mixed gently and then centrifuged (3200xG, 3 min, +4°C).
4. Samples were prepared using an automated 8-channel liquid handler (PerkinElmer JANUS Varispan Automated 8-tip Workstation) by mixing equal volumes (approximately 80 µL) serum and NMR measurement buffer (Na₂HPO₄ in 80%/20% H₂O/D₂O, pH 7.4; including also 0.08% sodium 3-(trimethylsilyl)propionate-2,2,3,3-d₄ and 0.04% sodium azide) as described in Soininen P. et al, 2015.
5. The prepared samples were measured using a Bruker AVANCE III HD 500 MHz spectrometer equipped with cooled robotic sample changer (SampleJet) and a cryogenically cooled triple resonance probe head (CryoProbe Prodigy TCI). The relevant NMR spectroscopy details are given in Soininen et al, 2009.

NMR data processing

1. The NMR data was processed, and metabolites were quantified using Nightingale Health's advanced proprietary software (Würtz et al, 2017).
2. The individual metabolite measurements and the overall metabolite distributions were analysed as part of Nightingale's quality control procedures with observations detailed in the results delivery for ADNI/Duke (ADMC Nightingale Platform-Report.pdf and ADCM Nightingale Platform-Result file description.pdf). Overall, the metabolite levels broadly fall within the distributions commonly observed in general population cohorts. The sample quality was deemed to be good.

Version Information

This document supersedes our previous document dated [17 July 2018]. Specific changes in our methods are summarized in this section.

Dataset Information

The September 2020 data was reanalyzed using the “2020 update”, an updated quantification library. This methods document applies to the following dataset(s) available from the ADNI repository:

Dataset Name	Date Submitted
ADMC Nightingale Platform NMR Analysis of Lipoproteins and Metabolites	29 September 2020
ADMC Nightingale Platform NMR Analysis of Lipoproteins and Metabolites Longitudinal	29 September 2020

References

1. Soininen P., Kangas A.J., Würtz P., Suna T., Ala-Korpela M. (2015) Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet* 8:192–206.

2. Soininen et al. (2009) High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst* 134:1781-1785.
3. Würtz et al, (2017) Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *Am J Epidemiol* 2017 186:1084-1096.

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