HOMOCYSTEINE (HCY) IN DRIED BLOOD SPOT (DBS)

General information

The laboratory-developed test (LDT) for quantitation of homocysteine (Hcy) utilizes capillary blood collected on the dried blood spot card (TFN, Ahlstrom-Munksjö). Test is based on isotope dilution tandem mass spectrometry methodology (ID LC-MS/MS).

Purpose

Measuring homocysteine (Hcy) concentrations in dried blood spots (DBS) serves as a reliable and efficient screening technique for identifying potential disturbances in homocysteine homeostasis, such as homocystinuria. Elevated homocysteine levels can be indicative of various underlying metabolic and genetic conditions, including deficiencies in vitamin B12, vitamin B6, and folic acid, or enzymatic defects related to methylation pathways.

Description

The test is based on a sequential extraction, reduction and derivatization followed by analysis using an a LC-MS/MS system. A two 3mm discs (~6,2 µl blood) are used for the extraction using mixture of homocystine stable isotope standard ($^2H_8$-homocystine) and disulfide bonds reduction solution (50mM TCEP). Extraction is stopped using protein precipitation reagent (acetonitrile) after incubation at a room temperature. Obtained extracts are evaporated and undergo derivatization using n-butanol in 3N HCl. Derivatized samples are injected into LC-MS/MS system, separated on reversed phase (RP) chromatographic column and analyzed using electrospray ion source (ESI) in multiple reaction monitoring (MRM) mode.

Assay is calibrated and controlled daily using commercially available DBS CAL and QC samples (Recipe MS2113, MS2182) and certified by external quality assurance program organized by ERNDIM (Special Assays in Dried Blood Spots - SADB).

Analytical and functional sensitivity

The sensitivity of homocysteine determination in dried blood spot, both analytical (LOD) and functional (LOQ), are calculated based on signal-to-noise (S/N) ratio of individual MRM transition. Linearity in dynamic range of method calibration is described by regression coefficient $R^2 \geq 0{,}970$.

	Analytical sensitivity (LOD, S/N ≥ 3)	Functional sensitivity (LOQ, S/N ≥10)	Upper Limit of Quantification (ULOQ)
Homocysteine DBS	0,4 µmol/l	2 µmol/l	44,8 µmol/l

Imprecision

An evaluation of the reproducibility of the DBS homocysteine assay was performed using quality control samples (ERNDIM). The study included samples at three concentration levels (QC1, QC2 and QC3) each in 5 replicates for 3 days (45 samples). The following tables provide representative data.

Sample	Mean conc. (µmol/l)	Intraday (within run)		Interday (total)	
		SD (µmol/l)	CV (%)	SD (µmol/l)	CV (%)
QC1	6,42	0,18	3%	0,45	7%
QC2	18,88	0,71	4%	0,79	4%
QC3	31,06	1,42	5%	1,24	4%

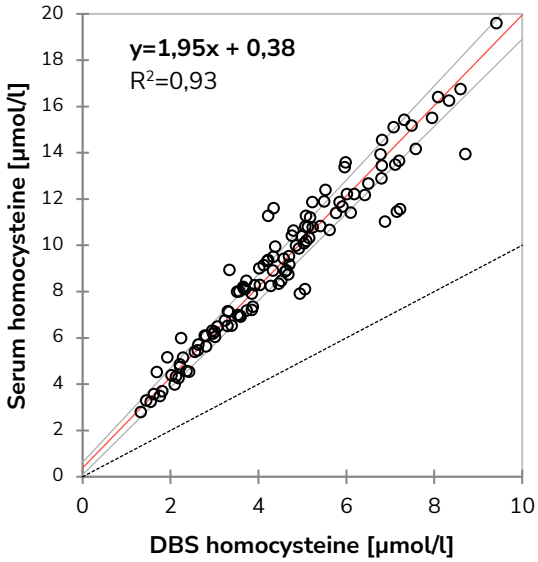
Recovery

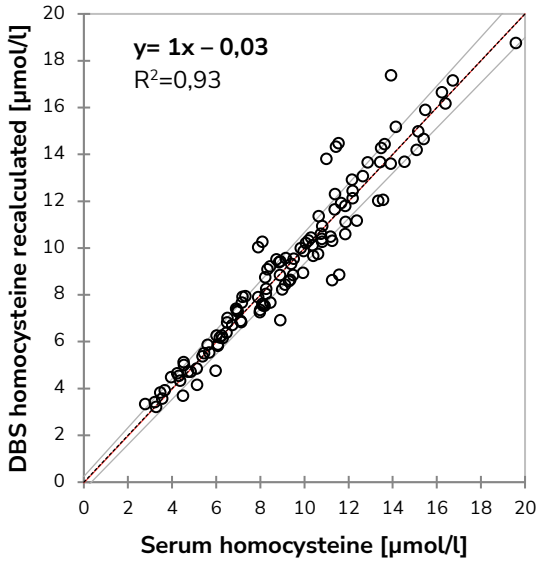
An evaluation of the homocysteine recovery in DBS homocysteine assay was performed using quality control samples (Recipe GmbH). The study included samples at three concentration levels (QC1, QC2 and QC3) each in 3 replicates. The following tables provide representative data.

Sample	Assigned conc. (µmol/l)	Mean conc. (µmol/l)	Recovery (%)
QC1	6,11	6,4	97%
QC2	10,2	10,13	89%
QC3	20,8	19,5	81%

Method comparison

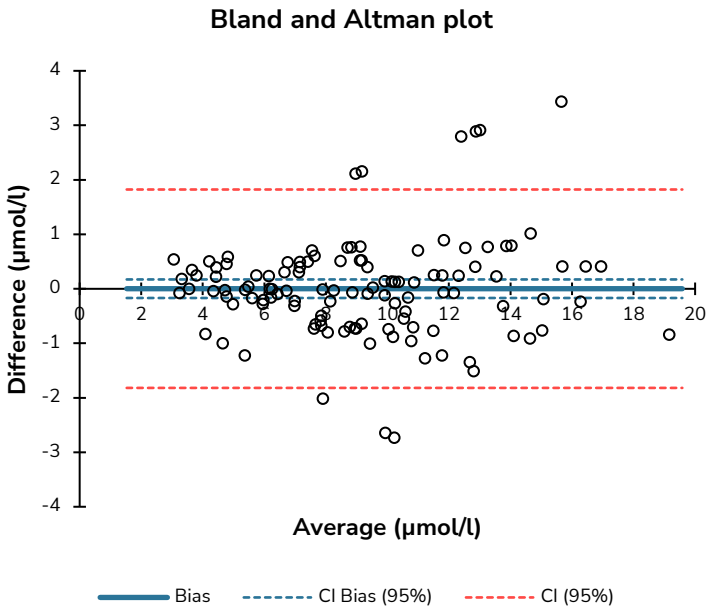
The analysis of method interchangeability between the reference method from serum and the developed method of capillary blood collection was carried out by analyzing samples on actual samples collected from patients in a clinical setting (n=117). Venous blood (serum clot activator) and fingertip capillary blood were collected simultaneously.





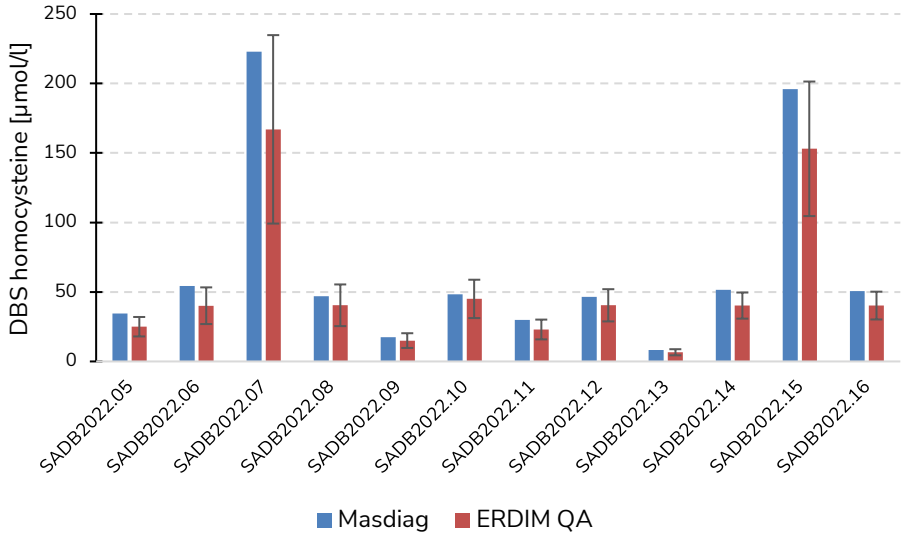
Analysis using Bland-Altman plot (XLSTAT Life Science v. 2023.1.4) of serum homocysteine against DBS results recalculated with regression coefficient y=1,95x + 0,38 yielded the bias between methods as follows (with 95% confidence intervals).

Constant bias: 0,002 µmol/l (CI95 between -0,17 µmol/l and 0,17 µmol/l) with 95% confidence intervals for method differences -1,82 µmol/l and 1,82 µmol/l.

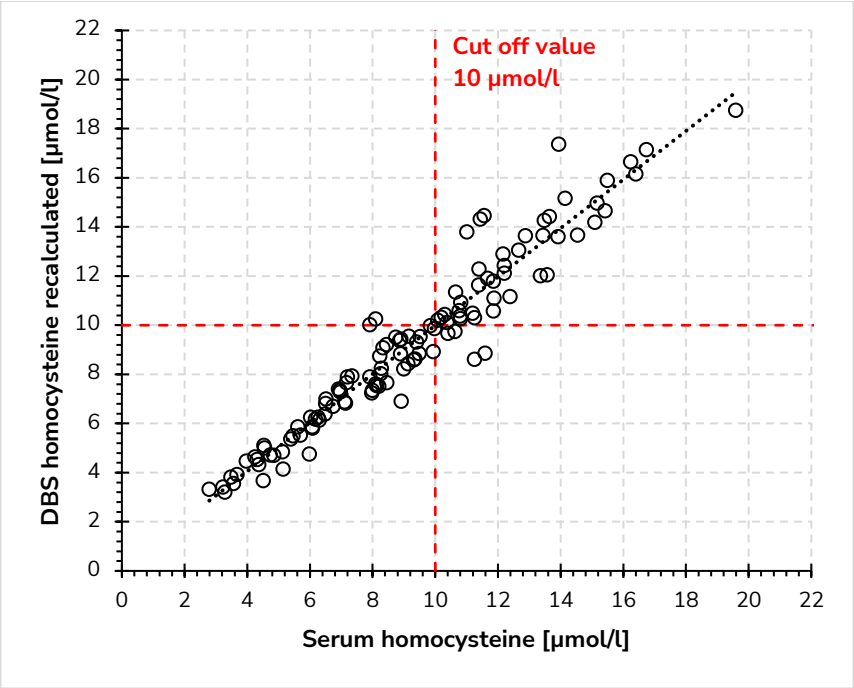


Diagnostic performance

The analysis of diagnostic performance of developed method was carried out based on ERDIM quality assurance program for homocysteine analysis in DBS (Special Assays in Dried Blood Spots - SADB).



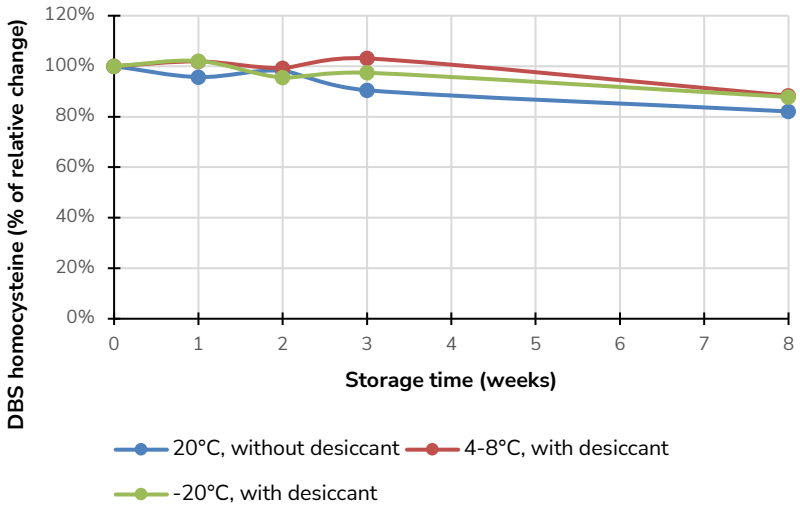
Diagnostic performance was assessed by analyzing homocysteine concentrations simultaneously using a serum reference method, as well as capillary blood samples collected from actual patients (n=117) in a clinical setting using the developed method of capillary blood collection. A simultaneous collection of venous blood (serum clot activator) and fingertip capillary blood was performed. Homocysteine DBS results were recalculated using regression parameters (x*1,95+0,38). The cut-off point value was established as 10 µmol/l.



		SERUM	
		Homocysteine ≥ 10µmol/l	Homocysteine < 10µmol/l
DBS recalculated	Homocysteine ≥ 10µmol/l	44	2
	Homocysteine < 10µmol/l	4	67

Sensitivity (TP/(TP+FN) = **91,7%**
Specificity (TN/(TN+FP) = **97,1%**

Storage, shipping and collection cards



Room temperature (18-25°C): up to 8 weeks
Refrigerated (2-8°C): up to 8 weeks
Frozen (-20°C): up to 8 weeks

Described method has proven performance using DBS collection cards modified with different preservatives:

- TNF unmodified card
- TFN card modified with FAPS (OmegaQuant)
- TFN card modified with FAPS without tocopherol (OmegaQuant)
- TFN card modified with BHT (LipidSaver)