

## Homocysteine (plasma, urine, dried blood spots)

### 1 Name and description of analyte

1.1 Name of analyte  
Homocysteine

1.2 Alternative names  
None

1.3 NLMC code  
To follow

1.4. Function(s) of analyte  
Homocysteine (HcyH) is a sulphur-containing amino acid ( $\text{HSCH}_2\text{CH}_2\text{CH}(\text{NH})\text{COOH}$ ). It is a metabolite of the methyl group donor S-adenosyl methionine, which is itself produced from methionine. There are two main pathways for metabolism of HcyH: it can be methylated to form methionine by methionine synthase, which requires 5-methyltetrahydrofolate (produced by enzymatic reduction of folate) and vitamin B<sub>12</sub> as cofactors; or it can be converted to cystathionine (which in turn can be converted to cysteine) by the enzyme cystathionine  $\beta$ -synthase (CBS), which requires vitamin B<sub>6</sub> as a cofactor.

Proposals for standardization of the nomenclature for homocysteine and related compounds were published in 2000. By these proposals HcyH is used to refer to the reduced molecule only. Other related compounds in plasma include: free homocystine (Hcy-Hcy, the oxidised dimeric form), mixed disulphides with other sulphhydryl compounds, for example cysteine, (Hcy-SR) and protein bound homocysteine (bHcy). The term total homocysteine (tHcy) refers to the homocysteine present in a sample following reduction of all disulphides.

### 2 Sample requirements and precautions

2.1 Medium in which measured  
EDTA or heparin plasma, urine, dried blood spots.

2.2 Precautions re sampling, handling etc.  
In unseparated blood kept at room temperature plasma [tHcy] increases by approximately 1  $\mu\text{mol/L/h}$ . This can be overcome by chilling of samples and performing centrifugation soon after collection or use of collection tubes containing 3-deazaadenosine as a stabiliser.

### 3 Summary of clinical uses and limitations of measurements

- 3.1 Uses
- As a risk factor for cardiovascular disease (CVD).
  - Diagnosis of homocystinuria and treatment monitoring.
  - Diagnosis and treatment monitoring of patients with folate or cobalamin deficiencies.

- Diagnosis of molybdenum cofactor deficiency and sulphite oxidase deficiency.

### 3.2 Limitations

An elevated plasma [tHcy] does not indicate the nature of the enzyme defect in patients with homocystinuria. Urine homocystine analysis has poor diagnostic sensitivity for the above disorders.

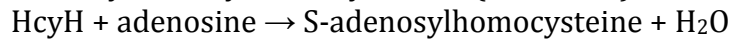
## 4 Analytical considerations

### 4.1 Analytical methods

All assays for measurement of [tHcy] rely on a reduction step to convert all homocysteine-related compounds to the reduced form.

#### 1. Immunoassay

This relies on the detection of S-adenosylhomocysteine (SAH), which is produced enzymatically from HcyH in a reaction catalysed by S-adenosylhomocysteine hydrolase (EC 3.3.1.1):

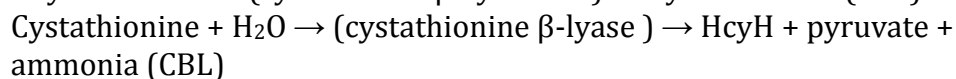
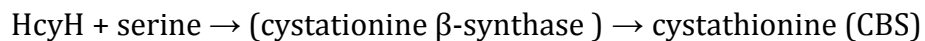


Following this enzymatic step, a monoclonal antibody is added to allow detection of SAH by competitive immunoassay. One approach uses fluorescence polarization to detect binding of antibody to a labelled SAH analogue. Alternatively, SAH from the sample competes for antibody binding with immobilised SAH, and the antibody bound to the immobilised SAH is measured using a labelled secondary antibody.

#### 2. Enzymatic

Cystathionine  $\beta$ -synthase (CBS; EC 4.2.1.22) coupled with cystathionine  $\beta$ -lyase (CBL; EC 4.4.1.8) and lactate dehydrogenase (LDH; EC 1.1.1.27).

The reactions are:



Consumption of NADH is measured spectrophotometrically at 340 nm.

#### 3. Immunonephelometry

This uses the same initial enzymatic step as the immunoassay methods to produce SAH. In the presence of SAH there is decreased aggregation of polystyrene bound anti-SAH antibodies by conjugated S-adenosylcysteine.

#### 4. HPLC

This uses either derivatisation of thiols with a fluorescent adduct and fluorescence detection, or electrochemical detection with no derivatisation.

#### 5. GC-MS

This method uses a derivatization step to form either tert-butyldimethylsilyl or *N(O,S)*-ethoxycarbonyl ethyl ester derivatives. An isotopic form of homocystine (homocystine- $d_8$ ) is commercially available for use as internal standard.

## 6. LC-MSMS

Homocysteine can be measured, without derivatization, by LC-MSMS in both plasma and urine. Homocysteine- $d_8$  is used as internal standard. An LC-MSMS method has also been described which allows detection of tHcy in dried blood spots. This method involves formation of butyl ester derivatives. Multiple SRM transitions are used to allow detection of [tHcy], other molecules of interest (for example methionine) and any internal standards used.

## 7. Amino acid analysis

An amino acid analyser using ion exchange chromatography with ninhydrin detection can be used to measure [tHcy], if a reduction step is used, or [Hcy-Hcy] if there is no reduction step.

The manual methods are more labour intensive and require handling of potentially harmful chemicals. The advantages of these manual methods are the potential for simultaneous detection of other relevant analytes (for example cysteine and methionine can also be detected by LC-MSMS). In addition, the measuring ranges of manual tests are in general wider (for example 0.2-300  $\mu\text{mol/L}$  and 0.2-200  $\mu\text{mol/L}$  have been quoted for GC-MS and LC-MSMS respectively compared with 2.0-64  $\mu\text{mol/L}$  for immunonephelometry, 0.4-50  $\mu\text{mol/L}$  for the enzymatic assay and 2.0-50  $\mu\text{mol/L}$  for immunoassay). Automated assays are therefore less suited to the diagnosis and monitoring of patients with homocystinuria, or for detecting the low levels of [tHcy] observed in sulphite oxidase deficiency. Studies published before the availability of a standard reference material suggested that results from HPLC, GC-MS and immunoassay methods did not show sufficient agreement to allow them to be used interchangeably. Urinary homocysteine can be measured by methods 4, 5, 6 and 7 but has been reported to show poor sensitivity for detection of hyperhomocysteinaemia (see section 8.1).

### 4.2 Reference method

Isotope dilution with GC-MS, LC-MS or LC-MSMS.

### 4.3 Reference materials

SRM 1955 (homocysteine and folate in human serum).

### 4.4 Interfering substances

The stabiliser 3 deaza-adenosine has been shown to produce negative interference with the immunoassay methods owing to inhibition of enzymatic conversion of HcyH to S-adenosylhomocysteine by S-adenosylhomocysteine hydrolase.

### 4.5 Sources of error

Delay in sample handling as mentioned (see 2.2).

## 5 Reference intervals and variance

### 5.1.1 Reference interval for (adults)

- [tHcy]  
 15-65 yr <15 µmol/L  
 > 65 yr <20 µmol/L
- 5.1.2 Reference intervals (others)  
 [tHcy]  
 Children <15 yr <10 µmol/L
- 5.1.3 Extent of variation  
 5.1.3.1 Interindividual CV: 40.3 %  
 5.1.3.2 Intraindividual CV: 9 %  
 5.1.3.3 Index of individuality; 0.28 (based on an analytical CV of 6.5 %)  
 5.1.3.4 CV of method:  
 Method dependent – values between 4.5 % and 8.5 % are quoted in the literature.
- 5.1.3.5 Critical difference:  
 31 % (based on an analytical CV of 6.5 %)
- 5.1.4 Sources of variation
- Plasma [tHcy] may increase 10–15 % 6–8 hours after a protein rich meal.
  - Values are around 10 % lower if a blood sample is collected when patient in the supine rather than sitting position.

## 6 Clinical uses of measurement and interpretation of results

### 6.1 Uses and interpretation

The main clinical uses of measurements of homocysteine are as follows.

- **Diagnosis of homocystinuria**  
 Plasma [tHcy] should be measured to aid diagnosis of homocystinuria in patients with suggestive symptoms. These symptoms include thromboembolism, lens dislocation, progressive myopia, osteoporosis, marfan-like features, unexplained mental retardation and megaloblastic anaemia. Renal failure and vitamin deficiency should be excluded as causes of severe hyperhomocystinaemia. The pathogenic defect can be determined by full blood count (to determine presence or absence of megaloblastic anaemia), measurement of folate, cobalamin, amino acids and transcobalamin in plasma, methylmalonic acid in urine as well as DNA analysis. Treatment depends on the defect and is monitored by measurement of plasma [tHcy] every 2–4 weeks until stable, then yearly. Following establishment of a diagnosis of homocystinuria siblings of the patient should also have plasma [tHcy] measured.
- **Diagnosis of folate or cobalamin deficiency**  
 Measurement of plasma [tHcy] has been suggested as a first line test for cobalamin or folate deficiency for patients considered at high risk or those with suggestive symptoms. A result within the reference range excludes deficiency in asymptomatic patients. Measurement of folate and cobalamin should follow if a high [tHcy] result (or high-normal result in a symptomatic patient) is obtained. If elevated plasma [tHcy] is not accompanied by low plasma [folate] or [cobalamin] then another cause for the elevated plasma [tHcy] should be sought. In deficient patients receiving replacement treatment, plasma [tHcy] should be measured 2–4 weeks after the beginning of treatment, then yearly if no symptoms arise.

If the plasma [tHcy] is unresponsive to treatment it suggests that treatment is incorrect or insufficient.

- **Cardiovascular disease**  
Raised plasma [tHcy] is an independent risk factor for vascular disease and it has been suggested that [tHcy] levels are a better predictor of recurrent, rather than primary, cardiovascular events. However [tHcy] adds little to predictions of vascular risk and therefore has no routine place in investigation or management of vascular disease risk or established disease. Moreover a clear cardiovascular benefit of lowering plasma [tHcy] (using folic acid and B vitamins) in the general population has not been demonstrated. Therefore, it does not appear that elevated plasma [tHcy] is a causal factor in development of vascular disease. The exceptions to this are homocystinuria patients, in whom the severe elevations of plasma [tHcy] are thought to be directly involved in thromboembolic events (due to the prothrombotic effects of HcyH). For this reason, measurement of plasma [tHcy] should be carried out in young CVD patients (< age 40) to exclude homocystinuria.
- **Diagnosis of molybdenum cofactor deficiency and sulphite oxidase deficiency**  
It has been suggested that that plasma [tHcy] should be measured as an initial screening test for molybdenum cofactor deficiency and sulphite oxidase deficiency in infants with intractable seizures or abnormal movements of uncertain cause. Plasma [tHcy] < 2.0  $\mu\text{mol/L}$  should prompt additional diagnostic tests (measurement of urate and sulphites in serum and urinary thiosulphate, S-sulphocysteine and oxypurine).

## 6.2 Confounding factors

Various drugs and other conditions can affect [tHcy]; see 7.1 and 7.2 for details.

## 7 Causes of abnormal results

### 7.1 High values

#### 7.1.1 Causes

- **Genetic causes**  
The term homocystinuria is used to refer to genetic causes of raised plasma and urine [tHcy]. Plasma [tHcy] may exceed 100  $\mu\text{mol/L}$  in these conditions. Homocystinuria encompasses the following disorders:
  - classic homocystinuria, which is caused by mutations in the enzyme cystathionine  $\beta$ -synthase (CBS) and is the commonest cause of homocystinuria. Worldwide, this condition affects 1 in 300 000 live births. There are pyridoxine responsive and non-responsive forms of this condition. The pyridoxine responsive form is the milder condition and the two forms may be distinguished by measuring the response of plasma [methionine] or [tHcy] to a pyridoxine challenge.
  - defective methylation of HcyH to produce methionine. This may be the result of mutations affecting methionine synthase, methylenetetrahydrofolate reductase or the metabolism and

transport of cobalamin. Certain cobalamin pathway defects are also associated with raised plasma [methylmalonic acid].

- milder increases in plasma [tHcy] may also be the result of inherited mutations such as heterozygosity for CBS mutations and homozygosity for the MTHFR 677 C→T polymorphism.
- Nutritional causes  
Elevated plasma [tHcy] can occur secondarily to deficiency of folate, cobalamin or vitamin B<sub>6</sub>. Cobalamin deficiency can result in plasma [tHcy] of >100 μmol/L; in folate deficiency plasma [tHcy] is likely to be < 100 μmol/L, and vitamin B<sub>6</sub> deficiency is associated with plasma [tHcy] of up to 30 μmol/L.
- Drugs  
There are several mechanisms whereby drugs can cause elevated plasma [tHcy]:
  - folate antagonism (methotrexate, trimethoprim).
  - cobalamin antagonism (nitrous oxide).
  - vitamin B<sub>6</sub> antagonists (niacin, azauridine, theophylline).
  - stimulating HcyH production (L-Dopa)
  - renal impairment (ciclosporin A, fibrates).These drugs can cause an elevation of plasma [tHcy] to up to up to 30 μmol/L, with the exception of nitrous oxide which can cause elevations of plasma [tHcy] of up to 100 μmol/L.
- Other causes:
  - hypothyroidism (up to 30 μmol/L)
  - renal failure (up to 100 μmol/L)
  - high alcohol consumption (above 100 μmol/L).

### 7.1.2 Investigation

Measurement of homocysteine should only be undertaken for the purposes indicated in 6.1. When an elevated value is found, renal function, the drug history and nutritional status should be investigated to eliminate incidental causes.

## 7.2 Low values

### 7.2.1 Causes

- Molybdenum cofactor deficiency and isolated sulphite oxidase deficiency  
Molybdenum cofactor (MoCo) is required for activity of four enzymes, including sulphite oxidase. MoCo deficiency can be caused by mutations in genes encoding any of the three enzymes required for its synthesis. This condition displays identical clinical features to isolated sulphite oxidase deficiency (infantile epileptic encephalopathy, progressive psychomotor retardation, severe microcephaly and later lens dislocation). Plasma [tHcy] has been reported to be undetectable in molybdenum cofactor deficiency and isolated sulphite oxidase deficiency. Reactive sulphite accumulation is a feature of these conditions and it has been suggested that this causes degradation of thiol containing compounds. The use of plasma [tHcy] as an initial screening test for this condition is covered in section 6.1.

- Other causes
  - pregnancy
  - hyperthyroidism
  - high intake of folate and B vitamins.
- Drugs
  - betaine
  - statins (lowering of [tHcy] has been demonstrated for simvastatin and atorvastatin)
  - *N*-acetylcysteine
  - tamoxifen.

### 7.2.2 Investigation

Serum urate and sulphites, and urinary thiosulphate, S-sulphocysteine and oxypurine should be measured if [tHcy] is < 2.0 µmol/L and the clinical features are consistent with molybdenum cofactor deficiency or sulphite oxidase deficiency.

### 7.3 Notes

None

## 8 Performance

### 8.1 Sensitivity, specificity etc. for individual conditions

Measurement of free homocystine has been reported to have poor sensitivity for hyperhomocysteinaemia. In order for free homocystine to be reliably detected in plasma or urine, concentrations need to be >60 µmol/L and 150 µmol/L, respectively. Measurement of urinary [tHcy] has been shown to have a similarly poor sensitivity to urinary [Hcy-Hcy] for detection of hyperhomocysteinaemia.

## 9 Systematic reviews and guidelines

### 9.1 Systematic reviews

None identified

### 9.2 Guidelines

1. Bowron A, Brown A, Deverell D *et al.* Metbionet guidelines for amino acid analysis. 2012. <http://www.metbio.net/metbioGuidelines.asp> [last accessed 21/09/2012].

2. British Cardiac Society, British Hypertension Society, Diabetes UK *et al.* JBS 2: Joint British societies guidelines on prevention of cardiovascular disease in clinical practice. *Heart*. 2005;91:V1-V52.

3. Myers GL, Christenson RHM, Cushman M *et al.* National Academy of Clinical Biochemistry laboratory medicine practice guidelines: emerging biomarkers for primary prevention of cardiovascular disease. *Clin Chem*. 2009;55:378-384.

### 9.3 Recommendations

1. Refsum H, Smith AD, Ueland PM *et al.* Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem.* 2004;50:3–32.

2. Mudd SH, Finkelstein JD, Refsum H *et al.* Homocysteine and its disulphide derivatives: a suggested consensus terminology. *Arterioscler Thromb Vasc Biol.* 2000;20:1704-1706.

## 10 Links

### 10.1 Related analytes

None

### 10.2 Related tests

Folate, cobalamin, methylmalonic acid, amino acids (specifically methionine and cysteine).

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