

Homocysteine enzymatic

In vitro test for the quantitative determination of total L-homocysteine in human serum and plasma

Indication

Homocysteine (Hcy) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Total homocysteine (tHcy) represents the sum of all forms of Hcy including forms of oxidized, protein-bound and free. Elevated levels of tHcy has emerged as an important risk factor in the assessment of cardiovascular disease. Excess Hcy in the blood stream may cause injuries to arterial vessels due to its irritant nature, and result in inflammation and plaque formation, which may eventually cause blockage of blood flow to the heart. Elevated tHcy levels are caused by four major factors, including:

- genetic deficiencies in enzymes involved in Hcy metabolism such as cystathionine beta-synthase (CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR)
- nutritional deficiency in B vitamins such as B6, B12 and folate
- renal failure for effective amino acid clearance
- drug interactions, such as with nitric oxide, methotrexate and phenytoin that interfere with Hcy metabolism. Elevated levels of tHcy are also linked with Alzheimer's disease and osteoporosis

Test principle: Enzyme cycling method

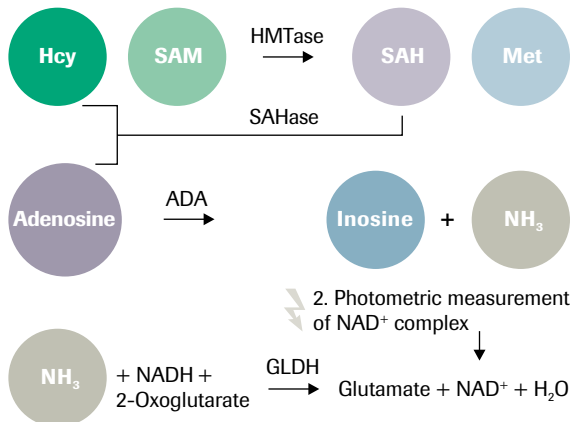
Step 1



1. Step

Oxidized Hcy that is bound to protein is first reduced to free Hcy.

Step 2



2. Step

Hcy then reacts with a co-substrate, S-adenosylmethionine (SAM), to form methionine (Met) and S-adenosyl homocysteine (SAH), catalyzed by a Hcy S-methyl transferase (HMTase). SAH is assessed by coupled enzyme reactions where SAH is hydrolyzed into adenosine and homocysteine by SAH hydrolase, and homocysteine is cycled into the homocysteine conversion reaction to form a reaction cycle that amplifies the detection signal. The formed adenosin is immediately hydrolyzed into inosine and ammonia (NH₃), which reacts with glutamate dehydrogenase (GLDH) with concomitant conversions of NADH to NAD⁺.

Photometric measurement

The concentration of Hcy in the sample is indirectly proportional to the amount of NADH converted to NAD⁺ ($\Delta A_{340\text{nm}}$).



Life needs answers

Homocysteine test characteristics

	Homocysteine enzymatic	Homocysteine enzymatic	Homocysteine enzymatic																		
Analyzer compatibility	cobas c 311 analyzer cobas c 501/ cobas c 502 module COBAS INTEGRA® 400 plus analyzer COBAS INTEGRA® 800 analyzer	cobas c 701/ cobas c 702 module	Roche/Hitachi MODULAR ANALYTICS <P>																		
Sample material	Serum, Plasma	Serum, Plasma	Serum, Plasma																		
Reaction time	10 minutes	10 minutes	10 minutes																		
Measuring range	3–50 µmol/L	3–50 µmol/L	3–50 µmol/L																		
On-board stability	4 weeks	4 weeks	4 weeks																		
Calibration frequency	Each reagent lot and after 7 days	Each reagent lot and after 7 days	Each reagent lot and after 7 days																		
Cystathionine interference	No cystathionine interference up to 100 µmol/L																				
Expected values ¹	<p>In most of the U.S. clinical laboratories, 15 µmol/L is used as the cut-off value for normal levels of Hcy in adults. In European laboratories, 12 µmol/L is used as the cut-off value for normal levels of Hcy in adults.</p> <p>Age, pregnancy, and renal function are important. The intake of folic acid as either supplements or through fortification of foods must also be considered:</p> <table border="1"> <thead> <tr> <th>Group</th> <th>Folate supplemented µmol/L</th> <th>Non supplemented µmol/L</th> </tr> </thead> <tbody> <tr> <td>Fasting/basal tHcy:</td> <td></td> <td></td> </tr> <tr> <td>Pregnancy</td> <td>8</td> <td>10</td> </tr> <tr> <td>Children < 15 years</td> <td>8</td> <td>10</td> </tr> <tr> <td>Adults 15-65 years</td> <td>12</td> <td>15</td> </tr> <tr> <td>Elderly > 65 years</td> <td>16</td> <td>20</td> </tr> </tbody> </table> <p>Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.</p>			Group	Folate supplemented µmol/L	Non supplemented µmol/L	Fasting/basal tHcy:			Pregnancy	8	10	Children < 15 years	8	10	Adults 15-65 years	12	15	Elderly > 65 years	16	20
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Repeatability	cobas c 501 module 8.26 µmol/L = 2.0% 13.1 µmol/L = 1.8% 30.0 µmol/L = 1.4% 44.4 µmol/L = 2.0%	cobas c 701 module 6.15 µmol/L = 2.1% 16.9 µmol/L = 1.4% 23.3 µmol/L = 1.3%	Roche/Hitachi MODULAR ANALYTICS <P> 7.91 µmol/L = 2.2% 14.4 µmol/L = 1.4% 47.2 µmol/L = 1.3%																		
Intermediate precision	cobas c 501 module 8.26 µmol/L = 2.3% 13.1 µmol/L = 2.1% 30.0 µmol/L = 1.8% 44.4 µmol/L = 2.2% Results for intermediate precision were obtained on the master system, the cobas c 501 module.																				

Order information

Homocysteine cobas c , INTEGRA	100 tests	05 385 415 190
Homocysteine cobas c 701/ cobas c 702 module	200 tests	06 542 921 190
Homocysteine Modular P	R1: 2 x 23 mL R2: 2 x 5 mL R3: 2 x 4 mL	05 385 377 190
HCYS Calibrator Kit	2 x 3 mL	05 385 504 190
HCYS Control Kit	Control 1: 2 x 3 mL Control 2: 2 x 3 mL	05 142 423 190

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Roche Diagnostics International Ltd
CH-6343 Rotkreuz
Switzerland
www.cobas.com

Reference

¹ Refsum H. Total Homocysteine: Guidelines for Determination in the Clinical Laboratory. *Clin Lab News* 2002; pp 2-14.