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 bloodstream 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**

1. **Step**  
   Oxidized Hcy that is bound to protein is first reduced to free Hcy.

   ![Enzyme cycling method](image)

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). The enzyme glutamate dehydrogenase (GLDH) catalyzes the reaction of ammonia with 2-oxoglutarate and NADH to form NAD⁺.

**Photometric measurement**  
The concentration of Hcy in the sample is directly proportional to the amount of NADH converted to NAD⁺ (ΔA₃₄₀nm).
Homocysteine test characteristics

<table>
<thead>
<tr>
<th>Analyzer compatibility</th>
<th>Homocysteine enzymatic</th>
<th>Homocysteine enzymatic</th>
<th>Homocysteine enzymatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>cobas c 311 analyzer</td>
<td>cobas c 701/cobas c 702 module</td>
<td>cobas c 701/cobas c 702 module</td>
<td>Roche/Hitachi MODULAR ANALYTICS &lt;P&gt;</td>
</tr>
<tr>
<td>cobas c 501/cobas c 502 module</td>
<td>COBAS INTEGRA® 400 plus analyzer</td>
<td>COBAS INTEGRA® 800 analyzer</td>
<td></td>
</tr>
</tbody>
</table>

Sample material
- Serum, Plasma
- 3–50 μmol/L
- 4 weeks
- Each reagent lot and after 7 days
- No cystathionine interference up to 100 μmol/L

Reaction time
- 10 minutes
- 10 minutes
- 10 minutes
- 10 minutes

Measuring range
- 3–50 μmol/L
- 3–50 μmol/L
- 3–50 μmol/L
- 3–50 μmol/L

On-board stability
- 4 weeks
- 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

Expected values
- 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.

Age, pregnancy, and renal function are important. The intake of folic acid as either supplements or through fortification of foods must also be considered:

<table>
<thead>
<tr>
<th>Group</th>
<th>Folate supplemented</th>
<th>Non supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting/basal tHcy:</td>
<td>μmol/L</td>
<td>μmol/L</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Children &lt; 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 &gt; 65 years</td>
<td>16</td>
<td>20</td>
</tr>
</tbody>
</table>

In European laboratories, 12 μmol/L is used as the cut-off value for normal levels of Hcy in adults.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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%

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 | 05 385 377 190 |
| | R2: 2 x 5 mL | |
| | R3: 2 x 4 mL | |
| HCYS Calibrator Kit | 2 x 3 mL | 05 385 504 190 |
| HCYS Control Kit | Control 1: 2× 3 mL | |
| | Control 2: 2× 3 mL | 05 142 423 190 |

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Reference