

New HemosIL® Homocysteine

*First Fully Automated Homocysteine Assay
for the Hemostasis Laboratory*

Hemostasis



*Streamline Thrombophilia Screening;
Complete Panel
on a Single Hemostasis System*

First Fully Automated Homocysteine Assay for the Hemostasis Laboratory

Homocysteine Facts

Homocysteine (Hcy) is derived from the metabolism of methionine, an essential amino acid in the diet. During metabolism, multiple methyl transfer reactions occur in most tissues, including vascular endothelial cells. Hcy is an intermediary in sulfur amino acid metabolism, linking the methionine cycle with the folate cycle. There are genetic and acquired defects that may affect the levels of Hcy in plasma. The table below summarizes Hcy, with a particular focus on Hyperhomocysteinemia.

Homocysteine in Plasma	<ul style="list-style-type: none"> • Mainly oxidized, with a relatively small portion in sulfidryl form (HcyH). • Hcy can be present in protein-bound (major fraction), non-protein-bound, or free-reduced form (minor fraction). • Total homocysteine (tHcy) is the sum of several circulating homocysteine species that can be measured in plasma or serum. tHcy plasma levels vary with age, gender, geographical area and genetic factors. • tHcy ranges for adults: 5-15
Hyperhomocysteinemia Definitions	<ul style="list-style-type: none"> • Moderate Hyperhomocysteinemia: fasting tHcy levels of 15-30 $\mu\text{mol/L}$. • Intermediate and Severe Hyperhomocysteinemia: fasting tHcy levels of 31-100 $\mu\text{mol/L}$ and 101-400 $\mu\text{mol/L}$.
Hyperhomocysteinemia Genetic Defects	<ul style="list-style-type: none"> • Elevated levels of Hcy may be caused by a homozygous or heterozygous deficiency of cystathionine-β-synthase (CBS). This results in normal fasting levels of Hcy, but substantially increased levels after methionine loading. • Genetic polymorphisms of the gene, methylene-tetrahydrofolate reductase (MTHFR), may also cause moderate hyperhomocysteinemia, particularly when folate levels are in the lower end of the normal range. The most common known mutations are MTHFR C677T and MTHFR A1298C. • Both CBS and MTHFR genetic defects cause homocystinuria.
Hyperhomocysteinemia Acquired Defects	<ul style="list-style-type: none"> • Patients with deficiencies of Vitamin B₁₂ or folate may demonstrate intermediate hyperhomocysteinemia. • Hyperhomocysteinemia caused by Vitamin B₆ deficiency may be detected particularly after methionine loading. • Other conditions leading to hyperhomocysteinemia include: renal insufficiency, hyperthyroidism or inflammatory bowel disease. Physical inactivity, smoking and caffeine may increase homocysteine plasma levels.
Hyperhomocysteinemia and Arterial Thrombosis	<ul style="list-style-type: none"> • Risk of cardiovascular disease increases 1.2 - 1.5 times for each 5 $\mu\text{mol/L}$ increment of Hcy. • The correlation is stronger with coronary heart disease than stroke. • Studies on the effect of lowering the tHcy with folic acid, Vitamin B₁₂ and Vitamin B₆ supplementation to reduce mortality and morbidity in patients with hyperhomocysteinemia are ongoing.
Hyperhomocysteinemia and Venous Thrombosis	<ul style="list-style-type: none"> • Hyperhomocysteinemia is an established risk factor for venous thrombosis (VTE). A recently published meta-analysis demonstrated that an increase of 5 $\mu\text{mol/L}$ tHcy corresponds to a 27% higher risk of VTE. • Another study demonstrated that the co-existence of hyperhomocysteinemia and Factor V Leiden mutation in apparently healthy men increases the risk of developing future VTE 20-fold. • Recent positions on the measurement of Hcy levels suggest testing individuals with idiopathic venous thrombosis, recurrent venous thrombosis, or venous thrombosis occurring at an early age or at an unusual site. • Vitamin supplements may be indicated for individuals with demonstrated folate or Vitamin B₁₂ deficiency. However, it is not yet known if lowering tHcy may reduce the risk of vascular disease.





HemosIL Homocysteine

HemosIL Homocysteine is the first fully automated Hcy assay dedicated to the Hemostasis laboratory.

- Fully automated and validated with Citrated Plasma on IL Hemostasis systems
- Solid and well-proven technology, derived from the widely used Elisa or Elisa-like methods
- Optimal analytical performances for the diagnosis of hyperhomocysteinemia (both fasting and after methionine loading)
- Allows for an extended thrombophilia work-up on a single coagulation system, in combination with other screening assays

Principle of the Assay

HemosIL Homocysteine is an automated latex enhanced immunoassay for the quantitative determination of total L-homocysteine in human citrated plasma. Hcy levels in patient plasma are measured automatically on IL Hemostasis systems in three stages:

- 1.Reduction of mixed disulfides and protein-bound forms of Hcy present in the plasma samples to free Hcy.
- 2.Enzymatic conversion of free Hcy to S-adenosyl-L-homocysteine (SAH) by the SAH hydrolase (SAHH) in the presence of an excess of adenosine. SAH can react with antibodies, but it has only one binding site.
- 3.SAH/conjugate has multiple binding sites, therefore allowing competitive immunoprecipitation reaction between anti-SAH latex and free SAH/conjugate.

The degree of agglutination is inversely proportional to the concentration of Hcy in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.

Reduction Step



Enzymatic Step



Competitive Agglutination Step

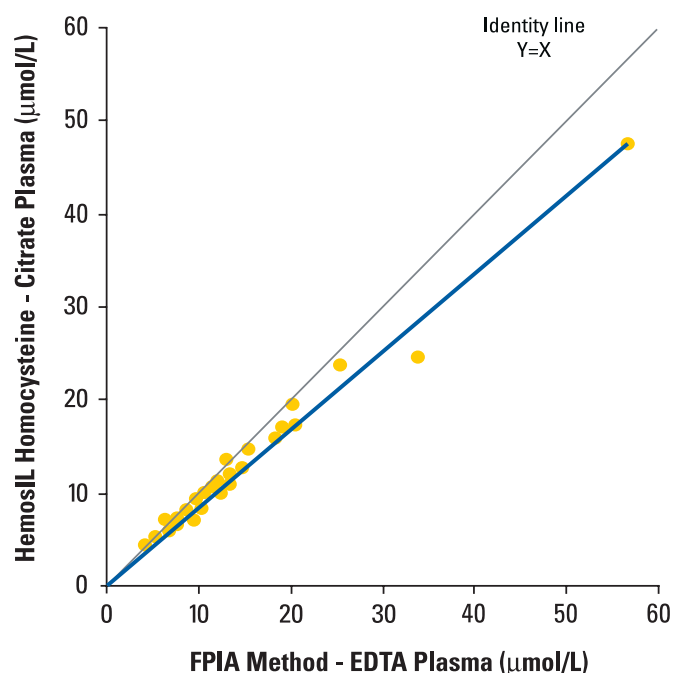


Measuring Homocysteine in Citrated Plasma Samples

HemosIL Homocysteine is adapted to ACL™ systems using citrated plasma samples. Citrated plasma yields lower Hcy results than EDTA and heparinized plasma due to the dilution with the anticoagulant. To convert Citrated plasma results to EDTA plasma results, multiply by 1.17. The sample preparation is a key factor among the pre-analytical variables for measuring Hcy. Following blood collection and before plasma separation centrifugation, there is a time and storage-temperature- dependent increase in tHcy that can be prevented either by centrifugation at 1000 x g for ten minutes within one hour; or, by keeping blood samples cooled on ice until centrifugation (up to eight hours). After the removal of red cells, tHcy is stable in plasma for at least three days at 2-8°C or three months at -20°C.

A method comparison between HemosIL Homocysteine on the ACL 9000 using citrated plasma samples versus Fluorescence Polarization Immunoassay (FPIA) using EDTA plasma demonstrates a very good correlation.

N: 76
 Slope: 0.8292
 Intercept: 0.3505
 R: 0.992



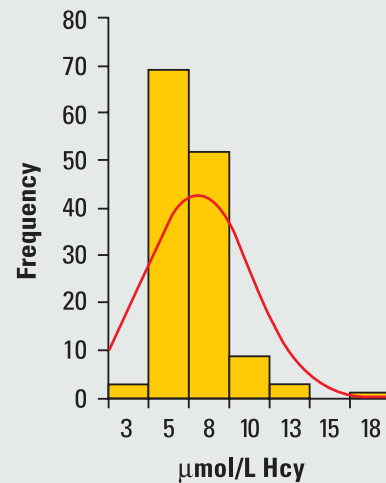
Assay Performance

The following data was obtained with a specific lot of reagent and is representative of average performance obtained on the ACL ELITE™ and ELITE PRO, ACL Futura/Advance and ACL TOP®.

Imprecision	Hcy levels	Total CV%
	~ 11 $\mu\text{mol/L}$	< 6%
	~ 22 $\mu\text{mol/L}$	< 7%
Detection Limit	2.4 $\mu\text{mol/L}$	
Linearity without Rerun	4.5 - 30 $\mu\text{mol/L}$	
Linearity with Automatic Rerun	up to 60 $\mu\text{mol/L}$	
On-board Reagent Stability	8 hours	

Normal Range Study on the ACL TOP

140 citrated plasmas, 50 males and 90 females, from apparently healthy blood bank donors, 18-65 years, were analyzed with the same lot of HemosIL Hcy on the ACL TOP. The upper limit of the normal range, corresponding to the 90% CI, was 11.1 $\mu\text{mol/L}$.



HemosIL Homocysteine and the Thrombophilia Work-up

Thrombophilia screening includes a number of tests for the identification of acquired or congenital risk factors.

The typical panel performed on the ACL Hemostasis systems include:

- Antithrombin
- Protein C
- Free Protein S Antigen
- Free Protein S Activity
- Factor V Leiden (APC R V)
- Lupus Anticoagulant

Other tests to detect Prothrombin G20210A mutation, elevated Factor VIII or dysfibrinogenemia may be included.

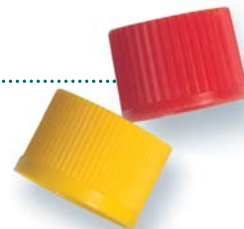
Homocysteine is a critical test for Thrombophilia screening the co-existence of hyperhomocysteinemia and Factor V Leiden mutation in apparently healthy men increases the risk of developing future VTE 20-fold.

Recent positions on the measurement of homocysteine levels suggest testing individuals with idiopathic venous thrombosis or recurrent venous thrombosis, or venous thrombosis occurring at early age or at unusual site.



New HemosIL Homocysteine

First Fully Automated Homocysteine Assay for the Hemostasis Laboratory



Reagent	Part Number	Kit Configuration	
Homocysteine Assay Kit	0020007800	<i>a</i> -SAH Latex Reagent	2 x 2 mL (lvo)
		Reductant	2 x 2 mL (liq)
		Enzyme	2 x 2 mL (liq)
		Conjugate	2 x 2.5 mL (liq)
		Buffer	2 x 9 mL (liq)
		Calibrator (30 µmol/L)	2 x 1 mL (liq)
Homocysteine Controls	0020007900	Level 1	3 x 1 mL (lvo) (~ 10 µmol/L)
		Level 2	3 x 1 mL (lvo) (~ 20 µmol/L)

References

- Den Heijer M. et al. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. *J Thromb Haemost* 2005; 3: 292-299.
- Refsum H. et al., Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem*, 2004; 50(1): 3-32.
- Martinelli I. et al., Hyperhomocysteinemia in cerebral vein thrombosis. *Blood* 2003 102(4): 1363 – 1366.
- Nigel S.K. et al., Hyperhomocysteinemia and Thrombophilia. *Arch Pathol Lab Med*, 2002; 126: 1367 – 1375.
- De Stefano V., et al., Screening for Inherited thrombophilia: indications and therapeutic implications. *Haematologica*, 2002; 87: 1095 – 1108.
- Tripodi A. and Mannucci PM., Laboratory Investigation of Thrombophilia. *Clin Chem*, 2001 47(9): 1597 – 1606.
- Cattaneo M. Hyperhomocysteinemia and thrombosis. *Lipids*, 2001; 36 Suppl: S13-26.
- Cattaneo M. Hyperhomocysteinemia and atherothrombosis. *Ann Med*, 2000; 32 Suppl 1:46-52.
- Westby C. et al., The stability of homocysteine concentration in blood samples. Poster presented at the IFCC, Florence, Italy 1999.
- Cattaneo M. Hyperhomocysteinemia: a risk factor for arterial and venous thrombosis. *Int J Clin Lab Res*, 1997; 27(3):139-44.
- Ridker PM et al., Interrelation of hyperhomocyst(e)inemia, Factor V Leiden, and risk of future venous thromboembolism. *Circulation*. 1997, 95(7):1777-82.
- Palmer-Toy D. et al. Compatibility of the Abbott IMx Homocysteine Assay with Citrate-Anticoagulated Plasma and Stability of Homocysteine in Citrated Whole Blood. *Clin Chem*, 2001; 47: 1704-1707.

Werfen Group IVD

Worldwide Locations

Corporate Headquarters

Barcelona, Spain
Tel. +34-93-4010101
www.chwerfen.com

Instrumentation Laboratory Headquarters

Lexington, MA
Tel. +1-781-861-0710
www.ilwww.com

US, Canada, Latin America

IL USA
Lexington, MA
Tel. +1-781-861-0710
www.ilus.com

IL Canada
Ontario, L4B 2N1
Tel. +1-800-522-2025x61115

IL Mexico
Col. Granada
Tel. +52-55-5262-1760
www.il-mexico.com.mx

Izasa Uruguay
Montevideo
Tel. +59-82-4818133

Pacific

Werfen Medical China
Shanghai
Tel. +86-21-32100745

Werfen Hong Kong
Hong Kong
Tel. +852-27927773

IL India
Janakpuri, New Delhi
Tel. +91-11-25510137

IL Japan
Minato-ku, Tokyo
Tel. +81-3-3437-6350

Werfen Medical Korea
Seo Cho-ku
Tel. +82-2-571-9246
www.werfenmedical.com

Europe, Middle East, Africa

Werfen Austria
Vienna
Tel. +43-1-2565800-0

IL Belgium
Zaventem
Tel. +32-2-7252052
www.il-be.com

Comesa Czech
Prague
Tel. +420-2-7816047

IL France
Paris
Tel. +33-1-53338600
www.il-france.fr

IL Germany
Munich
Tel. +49-89-909070
www.il-ger.de

IL Holland
Breda
Tel. +31-76-5480100
www.il-nl.com

Comesa Hungary
Budapest
Tel. +36-1-4392910 or 11

IL Italy
Milan
Tel. +39-02-25221
www.il-italia.it

IL Lithuania
Kaunas
Tel. +370-37-313157

Comesa Poland
Warsaw
Tel. +48-22-3361800

Izasa Portugal
Camaxide
Tel. +351-21-4247300
www.izasa.com

IL Russia
Moscow
Tel. +7-495-9823723

Izasa Spain
Barcelona
Tel. +34-93-4010101
www.izasa.com

IL UK
Warrington, Cheshire
Tel. +44-1925-81-0141
www.il-uk.com

Instrumentation Laboratory, Comesa and Izasa are Companies of Werfen Group IVD

Applications/tests listed may not yet be approved by the regulatory authorities in your country.
IL product specifications are subject to modification to assure the highest quality performance.
Some of the IL sites may still be in the process of completing ISO.
ACL and ACL Elite are trademarks and ACL TOP and HemosIL are registered trademarks of Instrumentation Laboratory.

© Instrumentation Laboratory 2006

