POINTE SCIENTIFIC
A MEDTEST COMPANY

Homocysteine Reagent

Intended Use

The Liquid Stable 2-Part Homocysteine Reagent is intended for *in vitro* quantitative determination of total homocysteine in human serum and plasma. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

Clinical Significance

Homocysteine (HCY) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Homocysteine is exported into plasma where it circulates, mostly in its oxidized form, bound to plasma proteins as a protein-HCY mixed disulfide with albumin (protein-SS-HCY).[1-5] Smaller amounts of reduced homocysteine and the disulfide homocystine (HCY-SS-HCY) are present. Total homocysteine (tHCY) represents the sum of all the HCY species found in serum or plasma (free plus protein bound). Homocysteine is metabolized to either cysteine or methionine. In the vitamin B6 trans-sulphuration pathway, homocysteine is irreversibly catabolized to cysteine. A major part of homocysteine is remethylated to methionine, mainly by the folate and cobalamin-dependent enzyme methionine synthase. Homocysteine accumulates and is excreted into blood when these reactions are impaired.[3,5] Severely elevated concentrations of total homocysteine are found in subjects with homocystinuria, a rare genetic disorder of the enzymes involved in the metabolism of homocysteine. Patients with homocystinuria exhibit mental retardation, early arteriosclerosis and arterial and venous thromboembolism.[2,6] Other less severe genetic defects which lead to moderately elevated levels of total homocysteine are also found.[7-9]

Epidemiological studies have investigated the relationship between elevated homocysteine levels and cardiovascular disease (CVD). A meta-analysis of 27 of these studies, including more than 4000 patients, estimated that a 5 µmol/L increase in total homocysteine was associated with an odds ratio for coronary artery disease (CAD) of 1.6 (95% confidence interval [CI], 1.4 to 1.7 for men and 1.8 (95% CI 1.3 to 1.9) for women; the odds ratio for cerebrovascular disease was 1.5 (95% CI 1.3 to 1.9). The risk associated with a 5 µmol/L increase in total homocysteine was the same as that associated with 0.5 mmol/L (20 mg/dL) increase in cholesterol. Peripheral arterial disease also showed a strong association.[10]

Hyperhomocysteinemia, elevated levels of homocysteine, can be associated with an increased risk of CVD. There have also been many published reports of prospective studies on the relationship between hyperhomocysteinemia and risk of CVD in men and women who were initially healthy. End points were based on a cardiovascular event such as acute myocardial infarction, stroke, CAD, or mortality. The results of eleven of these nested case-control studies reviewed by Cattaneo[11] were equivocal where five of the studies support the association with risk and six do not. More recently homocysteine levels were determined in a prospective study of post-menopausal women who participated in the Women's Health Study. Specimens from 122 women, who subsequently developed cardiovascular events, were tested for homocysteine and compared to a control group of 244 women who were matched for age and smoking status. The women in the control group remained free of disease during the three year follow-up period. The results demonstrated that post-menopausal women who developed cardiovascular events had significantly higher baseline homocysteine levels. Those with levels in the highest quartile had a two-fold increase in risk of any cardiovascular event. Elevated baseline homocysteine levels were shown to be an independent risk factor.[12] Also, homocysteine levels were determined in 1933 elderly men and women for the Framingham Heart Study cohort and demonstrated that elevated levels of homocysteine are independently associated with increased rates of all-cause and CVD mortality.[13]

Patients with chronic renal disease experience an excess morbidity and mortality due to arteriosclerotic CVD. Elevated concentration of homocysteine is a frequently observed finding in the blood of these patients. Although such patients lack some of the vitamins involved in the metabolism of homocysteine, the elevated HCY levels are mainly due to impaired HCY removal from the blood by the Kidneys.[14,15]

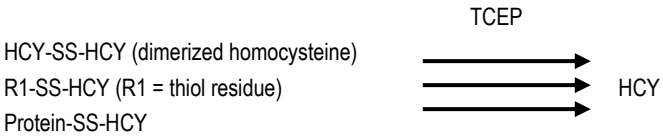
Recent evidence has also implicated elevated blood levels of homocysteine in miscarriages and birth defects.[16]

Drugs such as methotrexate, carbamazepine, phenytoin, nitrous oxide, and 6-azauridine triacetate interfere with HCY metabolism and may give elevated levels of HCY.[17]

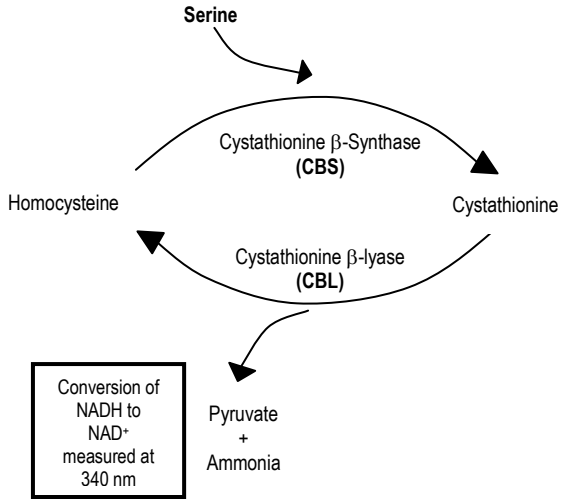
Test Summary and Principle

Bound or dimerized homocysteine (oxidized form) is reduced to free homocysteine, which then reacts with serine catalyzed by cystathionine beta-synthase (CBS) to form cystathionine. Cystathionine in turn is broken down by cystathionine beta-lyase (CBL) to form homocysteine, pyruvate and ammonia. Pyruvate is then converted by lactate dehydrogenase (LDH) to lactate with nicotinamide adenine dinucleotide (NADH) as coenzyme. The rate of NADH conversion to NAD^+ is directly proportional to the concentration of homocysteine (Δ A340 nm).

Reduction: Dimerized homocysteine, mixed disulfide, and protein-bound forms of HCY in the sample are reduced to form free HCY by the use of tris [2-carboxyethyl] phosphine (TCEP).



Enzymatic Conversion: Free HCY is converted to cystathionine by the use of cystathionine beta-synthase and excess serine. The cystathionine is then broken down to homocysteine, pyruvate and ammonia. Pyruvate is converted to lactate via lactate dehydrogenase with NADH as coenzyme. The rate of NADH conversion to NAD^+ (Δ A340 nm) is directly proportional to the concentration of homocysteine.



Reagents

R1 reagent: NADH (0.47 mM), LDH (38 KU/L), Serine (0.76 mM), Trizma Base 1-10%, Trizma Hydrochloride 1-10%, Sodium Azide < 1%. Reductant (TCEP:2.9 mM)
R2 reagent: Cycling Enzymes CBS (0.748 KU/L)and CBL (16.4 KU/L)
Sodium Azide < 1%.

Calibrator 1: Aqueous homocysteine blank (0 µmol/L).
Calibrator 2: Aqueous homocysteine solution (28 µmol/L).

Reagent Preparation
R1 and R2 are packaged ready to use. The reagents are stable until the expiration date specified on the label.

Indications of Deterioration
The reagents should be clear of particulate material. They should be discarded if they become turbid.

Calibrator Preparation and Use
The calibrators are prepared gravimetrically and are traceable to Standard Reference Material NIST SRM 1955, confirmed by a designated measurement procedure (HPLC). The calibrators are supplied in the kit and are provided ready to use. Values are printed on the labels.

Precautions
1. Adhere strictly to the instructions in this insert, particularly for handling and storage conditions.
2. Reagent 1 and Reagent 2 contain sodium azide which can react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large quantities of water to prevent azide build-up.
3. Material safety data sheets for all hazardous components contained in this kit are available upon request.

R22: Harmful if swallowed.
R32: Contact with acids liberates very toxic gas.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S29/35: Do not empty into drains; dispose of this material and its container in a safe way.
S46: If swallowed, seek medical advice immediately and show this container or label.

Reagent Storage
1. Store kit components at 2-8˚C and use until the expiry date on the labels. Do not use expired reagents.
2. Reagents may be used on multiple occasions until the expiry date on the labels. Reagents **must** be returned to 2-8°C storage between use.
3. Do not mix different reagent kit lot numbers.
4. **DO NOT FREEZE REAGENTS**.
5. Do not expose Reagent 1 and Reagent 2 to light during on-board use.
6. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.

Specimen Collection and Handling
1. Serum (collected in serum or serum separator tubes) and plasma (collected in potassium EDTA or lithium heparin tubes) may be used for the measurement of homocysteine.
   However, it is not recommended to use individual patient results from serum, heparinized plasma and EDTA plasma interchangeably.[27] Additionally matrix differences between serum and serum separator tubes and plasma tubes have been reported.[19]
   To minimize increases in homocysteine concentration from synthesis by red blood cells, process specimens as follows:
   - Place all specimens (serum and plasma) on ice after collection and prior to processing. Serum may clot more slowly and the volume may be reduced.[17]
   - All specimens may be kept on ice for up to 6 hours prior to separation by centrifugation.[17]
   - Separate red blood cells from serum or plasma by centrifugation and transfer to a sample cup or other clean container.

   Note: Specimens not placed on ice immediately may exhibit a 10-20% increase in homocysteine concentration.[18]
2. If the assay will be performed within 2 weeks after collection, the specimen should be stored at 2-8˚C. If the testing will be delayed more than 2 weeks, the specimen should be stored frozen at -20˚C or colder. Specimens have been shown to be stable at -20˚C for 8 months.[17,19]
3. It is the responsibility of the operator to verify the correct specimen type(s) is (are) used in the liquid stable 2-Part Homocysteine Reagent.
4. Inspect all samples (specimens, calibrators and controls) for bubbles. Remove bubbles prior to analysis.
5. Specimens containing particulate matter (fibrin, red blood cells, or other matter) and visibly lipemic specimens should not be used with the assay. Results from these specimens may be inaccurate.
6. Mix specimens **thoroughly** after thawing by low speed vortexing or by gentle inversion to ensure consistency in results. Avoid repeated freezing and thawing. Specimens showing particulate matter, erythrocytes, or turbidity should be centrifuged before testing.
7. On-board instrument storage. EDTA plasma samples can be stored for 3 hours on-board the AU400. The other recommended sample tubes for use on the assay have not been tested.

Materials Provided
Homocysteine Reagent R1 and R2, Calibrators

Materials Required But Not Provided
1. An analyzer capable of dispensing two reagents and of measuring absorbance at 340nm with temperature control (37°C).
2. Saline (0.85%)

Procedure (Automated) AU400® Procedure Parameters:

Test No. [*]	Name [HCY]	Type [Ser.]
Sample Volume:	[16.5] µL	Diluent Vo.l: [0.0] µL
Pre-Dilution Factor:	[1]	
Reagent 1 Volume:	[250] µL	Diluent Vol.: [0.0] µL
Reagent 2 Volume:	[25] µL	Diluent Vol.: [0.0] µL
Wavelength Pri:	[340] nm	
Wavelength Sec:	[380] nm	
Reaction Method:	RATE1	
Reaction Slope:	[-]	
Point 1	Fst [15]	Lst [27]
Point 2	Fst [ ]	Lst [ ]
Linearity	[100]%	
No-Lag-Time	[No]	
Min. OD	L [-2.0]	
Max. OD	H [2.5]	
Reagent OD Limit	Fst L [ ]	Fst H [ ]
	Lst L [ ]	Lst H [ ]
Dynamic Range:	L [1.0]	H [46.0]
Correlation Factor:	A [1.0]	B [0.0]
Onboard Stability Period:	[30]	

Calibration Specific:	Point	OD	Conc
	1 [*]	[ ]	[0.0]
	2 [*]	[ ]	[**]

Calibration Type:	[AA]
Formula:	Y=AX+B]

*User Defined
**Enter Values on Calibrator Vials
Ensure that the assay parameters exactly match those listed above.

Results
Results are printed out by the analyzer in µmol/L.

Limitations
1. The linear range of the liquid stable 2-Part Homocysteine Reagent when run as directed is 1-46 µmol/L. Specimens > 46 µmol/L should be diluted 1 part specimen to 2 parts Cal 0 µmol/L or 1 part specimen to 9 parts Cal 0 µmol/L as appropriate.
2. The Reagents should be clear. Discard if turbid.
3. Cystathionine is measured with homocysteine, but in the general population the cystathionine level (0.065 to 0.3 µmol/L) has a negligible effect. In very rare cases, end stage renal disease and patients with severe metabolic disturbances, cystathionine levels may rise dramatically and in severe cases cause greater than 20% interference.[25,26]
4. Hydroxylamine, present in several iron reagents may carryover (reagent probe or reaction cuvette) and cause falsely low results. Routine rinsing procedures are not adequate to eliminate this problem in most cases. Possible solutions would include special washing protocols, changing to an iron assay that used ascorbic acid as reductant or running iron and homocysteine assays on separate instruments.
5. Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6-azauridine triacetate may affect the homocysteine concentration.[17]
6. Samples with raised protein levels show > 10% difference compared to results obtained from normal samples and should be avoided.
7. Note: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azauridine triacetate, may have elevated levels of homocysteine due to their effect on the pathway.
8. Specimens containing particulate matter (fibrin, red blood cells, or other matter) and visibly lipemic specimens should not be used with the assay. Results from these specimens may be inaccurate.

Quality Control
Ensure that adequate maintenance and calibration is performed according to the manufacturer's instructions.

Assayed control materials with values for homocysteine in both the normal and abnormal ranges should be tested to validate reagent performance. Users should ensure that they are fully acquainted with the instructions for the assay, particularly the Precautions and the Reagent Storage sections. Users should demonstrate that they obtain performance specifications for precision and reportable range of test results comparable to those established by the manufacturer before reporting patient test results.

A Pointe Scientific Inc. Homocysteine Control Kit (H7575-CTL) containing low, medium and high controls is also available from Pointe Scientific Inc. for use with the liquid stable 2-Part Homocysteine Reagent.

Expected Values
Reference Range: The reference range should be determined by each laboratory to confirm the characteristics of the population being tested. As a point of reference the following data may be used until the laboratory has analyzed a sufficient number of specimens to determine its own reference range. The HCY concentration in plasma or serum of healthy individuals varies with age, gender, geographical area and genetic factors. Scientific literature reports reference values for adult male and females between 5 and 15 µmol/L, men having higher values than women, and post menopausal woman having higher homocysteine values than pre-menopausal women.[17,20,21] HCY values will normally increase with age, giving a reference range among an elderly population (> 60 years) of 5-20 µmol/L.[22] In countries with folic acid fortification programs, reduced levels of HCY may be observed.[23,24]

Measurable Range: The measurable range of the liquid stable 2-Part homocysteine assay is 1-46 µmol/L.

Performance Characteristics
Established on the AU400®:

Accuracy: A correlation study was performed with plasma specimens from apparently healthy adults. All specimens were analyzed using the Pointe Scientific, Inc. liquid stable Homocysteine Reagent and a similar liquid stable Homocysteine Reagent (Catch), according to the CLSI (formerly NCCLS) document EP9-A2.[28] All results are based on 95% confidence Interval. Pointe Scientific, Inc. Liquid stable Homocysteine Reagent specimens ranged from 6.5 to 49.0 µmol/L. The data obtained gave the following statistical values:

Comparison Method	*Pointe Scientific Inc. vs. Catch*
Number of specimens	94
Slope of regression line	0.991
Y-Intercept	0.165
Correlation coefficient	1.0

Precision: A study was performed with guidance from the CLSI (formerly NCCLS) Document EP5-A2.[29] Three HCY controls and three human plasma panels were assayed using two lots of reagents, in replicates of two, at two separate times per day for 20 days on one instrument (n=80). A calibration curve was generated at the start of the study and was used throughout. Results (*rounded to 1 decimal place*) are summarized below:

Sample	*Reagent Lot*	*n*	*Mean (µmol/L)*	*Within Run CV%*	*Total CV%*
Panel 1	*1*	*80*	*7.0*	*1.9*	*3.3*
	2	*80*	*7.0*	*2.2*	*4.4*
Panel 2	*1*	*80*	*36.0*	*1.3*	*2.5*
	2	*80*	*35.5*	*1.1*	*2.3*
Panel 3	*1*	*80*	*48.3*	*1.1*	*2.0*
	2	*80*	*47.7*	*1.0*	*2.2*
Low Control	*1*	*80*	*6.3*	*2.6*	*4.4*
	2	*80*	*6.3*	*2.1*	*4.1*
Medium Control	*1*	*80*	*12.3*	*1.5*	*3.0*
	2	*80*	*12.2*	*1.3*	*3.2*
High Control	*1*	*80*	*25.5*	*1.5*	*2.5*
	2	*80*	*25.3*	*1.6*	*2.9*

Dilution Linearity
The dilution linearity of the liquid stable 2-Part Homocysteine Reagent gives a % recovery range of 91–104% for all samples across the range of the assay (1–46 µmol/L) on the OLYMPUS AU400.

Samples > 46 µmol/L exhibit mean recovery of 100% ± 11% of the expected result when diluted into the assay range.

Limit of Detection
The limit of detection (LOD) of the liquid stable 2-Part Homocysteine Reagent according to the CLSI (formerly NCCLS) Document EP17-A[30] was found to be 0.33 µmol/L.

Analytical Specificity:
The specificity of the liquid stable 2-Part Homocysteine Reagent was assessed according to guidance in the CLSI Document EP7-A2[31] for the interfering substances listed in the following table:

Interfering Substance	*Interfering Substance Concentration*	*% Interference*
Bilirubin	20 mg/dL	≤ ±10
Haemoglobin	500 mg/dL	≤ ±10
Red Blood Cell	0.4%	≤ ±10
Triglyceride (Intralipid solution)	500 mg/dL	≤ ±10
Glutathione	1000 µmol/L	≤ ±10
Methionine	800 µmol/L	≤ ±10
Cysteine	200 µmol/L	≤ ±10
Pyruvate	1250 µmol/L	≤ ±10

None of these substances interfered significantly in the assay.

Samples with raised protein levels show > 10% difference compared to results obtained from normal samples and should be avoided.

Refer to References section of this product insert (ref 17) for possible interferences caused by drugs, disease or preanalytical variables.

References

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Store at 2-8°C

IVD *In Vitro* Diagnostic Medical Device

Consult Instructions For Use

Manufactured for
Pointe Scientific, Inc.

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