HCYS

Homocysteine Enzymatic Assay

cobas®

Substrates

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack can be used
05385415 190	Homocysteine Enzymatic Assay (100 tests)	System-ID 07 7487 1	COBAS INTEGRA 400 plus
05385504 190	HCYS Calibrator Kit (2 x 3 mL)	System-ID 07 7493 6	
05142423 190	HCYS Control Kit Control 1 (2 x 3 mL) HCYS Control Kit Control 2 (2 x 3 mL)	System-ID 07 7490 1 System-ID 07 7492 8	
20756350 322	NaCl Diluent 9 % (6 x 22 mL)	System-ID 07 5635 0	

English

For use in the USA only

Specimens from patients who are on drug therapy involving S-adenosylmethionine 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. Refer to the Limitations-interference section in this package insert.

System information

Test HCYS, test ID 0-006

Intended use

In vitro test for the quantitative determination of total L-homocysteine in human serum and plasma on COBAS INTEGRA systems. The assay can assist in the diagnosis of patients suspected of having hyperhomocysteinemia or homocystinuria.

Summary[1]

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 tHcy levels are caused by four major factors, including:

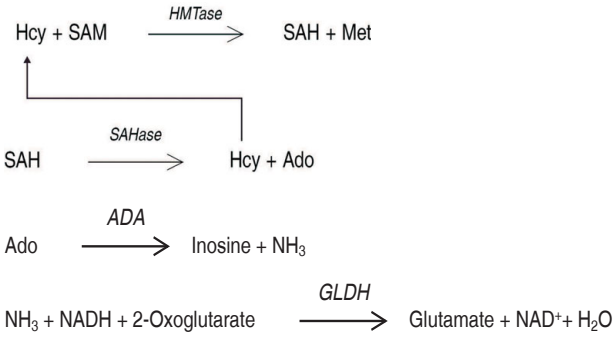
1. genetic deficiencies in enzymes involved in Hcy metabolism such as cystathionine beta-synthase (CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR);
2. nutritional deficiency in B vitamins such as B_6, B_{12} and folate;
3. renal failure for effective amino acid clearance; and
4. drug interactions, such as with nitric oxide, methotrexate and phenytoin that interfere with Hcy metabolism.

Excess Hcy is related to a higher risk of coronary heart disease, stroke, and peripheral vascular disease (fatty deposits in peripheral arteries). 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. Homocysteine levels may be reduced with treatment, but that does not necessarily reduce the occurrence of cardiovascular disease (CVD) events such as stroke and heart attack. Because findings from studies that evaluated the association between the increase in Homocysteine levels and CVD have been inconsistent, the American Heart Association has not yet called hyperhomocysteinemia (elevated Hcy in the blood) a major risk factor for CVD.[2]

Guidelines for tHcy determination in clinical laboratories have recently been established.[3,4]

Test principle

Homocysteine Enzymatic Assay is based on a novel enzyme cycling assay principle that assesses the co-substrate conversion product instead of assessing co-substrate or Hcy conversion products of Hcy. In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), to form methionine (Met) and S-adenosylhomocysteine (SAH), catalyzed by a Hcy S-methyltransferase. SAH is assessed by coupled enzyme reactions where SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase, and Hcy is cycled into the Hcy conversion reaction to form a reaction cycle that amplifies the detection signal. The formed Ado is immediately hydrolyzed into inosine and ammonia. In the last step, the enzyme glutamate dehydrogenase (GLDH) catalyzes the reaction of ammonia with 2-oxoglutarate and NADH to form NAD^+. The concentration of Hcy in the sample is directly proportional to the amount of NADH converted to NAD^+ ($\Delta A_{340\ nm}$).



Reagents - working solutions

R1 **NADH reagent**
S-adenosylmethionine 0.1 mmol/L; TCEP[a)] > 0.5 mmol/L; 2-oxoglutarate < 5.0 mmol/L; NADH > 0.2 mmol/L; buffer, pH 9.1 (25 °C); preservative; stabilizer

R2 **Enzyme reagent**
Homocysteine S-methyltransferase (HMTase) 5.0 kU/L; glutamate dehydrogenase (GLDH) 10 kU/L; casein (bovine) ≤ 0.2 %; buffer; pH 7.2 (25 °C); preservative; detergent

SR **Start reagent**
Adenosine deaminase (bovine) 5.0 kU/L; S-adenosyl-homocysteine hydrolase (SAHase) 3.0 kU/L; casein (bovine) ≤ 0.2 %; buffer, pH 7.2 (25 °C); preservative; stabilizer

a) Tris(2-carboxyethyl)phosphine

R1 is in position A, R2 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
On-board in use at 10-15 °C	4 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin, K_2-EDTA and K_3-EDTA plasma

It is important to centrifuge blood samples immediately after collection to separate the plasma from the blood cells. If immediate centrifugation is not possible, collected blood specimens should be kept on ice and centrifuged within an hour. Hemolysed samples should not be used for this assay. Turbid specimens or severely lipemic specimens are not recommended for the Hcy assay.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all

available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

See the limitations and interferences section for details about possible sample interferences.

Stability:[4,5,6] 4 days at 15-25 °C
4 weeks at 2-8 °C
10 months at -20 °C

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350 322, system-ID 07 5635 0 for *automatic postdilution and standard serial dilutions.* NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus analyzers.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for serum and plasma

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1/R2-S-SR
Reaction direction	Decrease
Wavelength A/B	340/659 nm
Calc. first/last	50/62
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
R1	175 µL	–
R2	27 µL	–
Sample	14 µL	–
SR	18 µL	–
Total volume	234 µL	

Calibration

Calibrators	HCYS Calibrator Kit Calibration dilution ratio: 1:1, 1:2, 1:4, 1:8, 1:18 performed automatically by the instrument
Calibration mode	Logit/log 5
Calibration replicate	Duplicate recommended
Calibration frequency	Full calibration • after 7 days on board • after reagent lot change • as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against NIST SRM 1955 reference material.

Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus:[7] No significant interference up to an I index of 20 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis:[7] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L or 100 mg/dL).

Lipemia (Intralipid):[7] No significant interference up to an L index of 250 (approximate Intralipid concentration: 250 mg/dL). There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Triglycerides: No significant interference from triglycerides up to a concentration of 2570 mg/dL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9] Additional drugs tested include Glutathione at 0.5 mmol/L, Cystathionine at 100 µmol/L, and Pyruvate at 0.5 mmol/L; no interference was found.

Note: Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate, may have higher levels of Hcy due to metabolic interference with Hcy metabolism.[3,6]

S-Adenosylhomocysteine (SAH) will cause a significant positive interference. However, SAH is only detectable at sub-nmol/L concentrations in normal plasma, and should not cause concern.[10]

Addition of 3-deazaadenosine to inhibit Hcy production in red cells has been suggested. However, the Homocysteine Enzymatic Assay cannot use samples containing 3-deazaadenosine since it inhibits one of the key enzymes used in the assay.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[11]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

3-50 µmol/L

The lower and the upper limit of the measuring range depends on the actual calibrator value.

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, (LoB) and Limit of Detection (LoD)

Limit of Blank = 0.63 µmol/L

Limit of Detection = 1.40 µmol/L

Results for LoB and LoD were determined on the COBAS INTEGRA 800 analyzer and met the predetermined specification of ≤ 3 µmol/L for both LoB and LoD.

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

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.[4]

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

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	*Mean µmol/L*	*SD µmol/L*	*CV %*
Homocysteine Control 1	12.2	0.1	1.0
Homocysteine Control 2	38.9	0.5	1.3
Human serum 1	8.47	0.09	1.1
Human serum 2	13.5	0.1	0.9
Human serum 3	31.2	0.3	0.9
Human serum 4	45.5	0.6	1.4

Intermediate precision	*Mean µmol/L*	*SD µmol/L*	*CV %*
Homocysteine Control 1	12.2	0.2	1.4
Homocysteine Control 2	38.9	0.6	1.5
Human serum 1	8.47	0.11	1.3
Human serum 2	13.5	0.2	1.4
Human serum 3	31.2	0.5	1.4
Human serum 4	45.5	0.8	1.7

Method comparison

Hcy values for human serum samples obtained on a COBAS INTEGRA 800 analyzer (y) were compared with those determined using the Diazyme reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 57

Passing/Bablok[12]	Linear regression
y = 1.00x + 0.144 µmol/L	y = 1.04x - 0.224 µmol/L
τ = 0.967	r = 0.998

The sample concentrations were between 3.39 and 46.8 µmol/L.

References

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8 Breuer J. Report on the Symposium “Drug effects in Clinical Chemistry Methods”. Eur J Clin Chem Clin Biochem 1996;34:385-386.

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12 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336