**CHEM.CENTAUR.ASSAY.21.0 Homocysteine by ADVIA Centaur**

**PRINCIPLE**

Homocysteine (HCY) is a naturally occurring amino acid that is formed from methionine as a product of numerous S-adenosylmethionine-dependent transmethylation reactions. The metabolism of HCY is regulated by three enzymatic pathways that either convert HCY into cysteine or remethylate it back into methionine. Homocysteine readily forms disulfide bonds and is present in plasma in three forms: free or unbound HCY (1 to 2%), homocysteine-cysteine or homocystine dimers (10 to 20%), or protein bound (>80%). Total plasma HCY, free and bound is commonly referred to as either homocysteine or homocyst(e)ine.

If one or more of the HCY metabolic pathways are inhibited due to enzymatic defects or vitamin deficiencies, HCY accumulates, causing an increased HCY level in plasma. Homocysteinuria is a rare group of genetic diseases where a deficiency in one of the HCY regulating enzymes results in a high plasma HCY levels and HCY excretion in urine. Individuals who are homozygous for one of the enzyme deficiencies will exhibit hyperhomocysteinemia. Deficiencies in either folic acid, vitamin B6, or vitamin B12 can produce hyperhomocysteinemia. Other studies show that chronic renal failure is also associated with elevated HCY levels.

A relationship between homocysteinuria and the development of premature arteriosclerotic disease was first observed over 30 years ago. More recently, several clinical and epidemiological studies have indicated that even a moderately elevated plasma HCY level is a predictor for cardiovascular disease.

The ADVIA Centaur HCY assay is a competitive immunoassay using direct chemiluminescent technology.

The system automatically performs the following steps:

* Dispenses 20 µl of sample into a cuvette.
* Dispenses 50 µl of Reducing Reagent and incubates for 3.0 minutes at 37°C.
* Dispenses 50 µl of Enzyme Reagent and incubates for 2.5 minutes at 37°C.
* Dispenses 250 µl of Acid Solid Phase and incubates for 2.5 minutes at 37°C.
* Dispenses 100 µl of Lite Reagent and incubates for 2.5 minutes at 37°C.
* Separates, aspirates, and washes the cuvettes with reagent water.
* Dispenses 300 µl each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
* Reports results according to the selected option, as described in the system operating instructions or the online help system.

An inverse relationship exists between the amount of HCY present in the patient sample and the amount of relative light units (RLUs) detected by the system.

**DOCUMENT OWNER**

Manager, Chemistry & Special Chemistry

**RELATED DOCUMENTS**

CHEM.CENTAUR.2.0 ADVIA Centaur XP Operating Procedure

CHEM.CENTAUR.2.1 Centaur Dilutions and Notes for Autofile and Miscellaneous Notes

**SPECIMEN**

1. Specimen Requirements:

1. Plasma (EDTA or Lithium Heparin)

a. Preferred specimen

2. Serum

1. Specimen Stability:

1. Refrigerated (2-8°C): 2 days (48 hours)

2. Frozen (-20°C): 13 weeks

1. Minimum volume: 80 µl
2. Special instructions

1. Centrifuge samples and remove serum or plasma from the red blood cells as soon as possible to ensure accurate measurement.

2. Samples that cannot be separated soon after collection should be stored on ice until centrifugation.

3. Samples should be free of fibrin or other particulate matter.

4. Samples should be free of bubbles.

**MATERIAL**

A. Controls

**REAGENTS**

* 1. ADVIA Centaur HCY ReadyPack primary reagent pack
  2. Lite Reagent (10.0 ml). Contains monoclonal mouse anti-SAH antibody (~4ug/mL) labeled with acridinium ester in phosphate buffer with bovine serum albumin preservatives.

a. Reagents are stable until the expiration date on the pack label

b. Stored at 2-8°C.

c. Onboard stability 28 days.

d. Calibration interval 14 days.

* 1. Solid Phase (25 ml). SAH (~2.1 ug/ml) covalently coupled to paramagnetic particles in phosphate buffered saline with bovine serum albumin and preservatives.

a. Reagents are stable until the expiration date on the pack label

b. Stored at 2-8°C.

c. Onboard stability 28 days.

d. Calibration interval 14 days.

* 1. Enzyme Reagent contains 5 ml bovine derived S-adenosylhomocysteine hydrolase enzyme (~60 mU/mL) in TRIS buffer with preservatives.

a. Reagent is stable until the expiration date on the pack label

b. Stored at 2-8°C.

c. Onboard stability 28 days.

d. Calibration stability 14 days.

* 1. ADVIA Centaur HCY Ancillary ReadyPack.

1. Reducing Reagent contains 10 ml dithiothreitol (~1.5 mg/ml) in citrate buffer with preservatives.

a. Reagent is stable until the expiration date on the pack label or 41 consecutive days after accessing the ancillary pack.

b. Stored at 2-8°C

1. HCY Diluent contains 10 ml phosphate buffer with bovine gamma globulin and preservatives.

a. Store at 2-8°C.

b. Stable until the date on the pack label or 41 consecutive days after accessing the ancillary pack.

**EQUIPMENT**

ADVIA Centaur XP

**CALIBRATION**

A. Homocysteine uses Calibrator HCY

B. The ADVIA Centaur HCY assay requires a Master Curve calibration:

1. Every 14 days
2. When changing lot numbers of primary reagent packs
3. When replacing system components
4. When quality control results are repeatedly out of range

C. For each new lot number of Lite Reagent and Solid Phase, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For Detailed information about entering calibrating values, and performing a calibration, refer to system operating instructions or to the online help system.

**QUALITY CONTROL**

A minimum of two levels of controls spanning the medical decision range are to be run once every 24 hours of assay use. See QC procedure CHEM.QC.REGCHEM.2.0 for specific details.

**PROCEDURE**

See ADVIA operating procedure CHEM.CENATUAR.2.0, for a detailed description of how to operate the applicable ADVIA Centaur instrument.

**DILUTION**

A. Specimens with homocysteine values exceeding 65 µmol/L shall be diluted.

B. Automated Dilution

1. First automated dilution is 1:2; program a 1:10 dilution if needed

2. Ensure that the ADVIA Centaur HCY Diluent is loaded and set the system parameters as follows:

a. Dilution point: ≤65 µmol/L

b. Dilution factor: 2, 10

C. Manual Dilution

1. If a physician requests a dilution to endpoint, make a manual dilution using HCY Diluent. Attach coded comment PRDIL to result.

2. If a dilution factor is entered when scheduling the HCY test, the system will automatically calculate the result.

D. Specimens with homocysteine values exceeding 650 µmol/L shall be reported as >650 µmol/L.

F. Specimens with homocysteine values below 0.5 µmol/L shall be reported as <0.5 µmol/L.

**CALCULATIONS**

N/A

**REPORTING RESULTS**

A. Normal Range:

Male <11.4 µmol/L

Female <10.4 µmol/L

B. Critical value: None

C. Linearity: 0.5 – 65 µmol/L

D. Extended Linearity: 0.5 – 650 µmol/L

**RESULT ENTRY**

1. Sunquest Computer Entry
2. Manual Entry

Function: MEM

Worksheet: CI

Method Code: CI1

Test Code: HCYST

2. Online Entry

Function: OEM

Device: CI1

Test Code: HCYST

1. QLS Computer Entry
2. Enter: 3, 3, 1
3. Worklist: CIM15
4. Test Code: 31789
5. Accession number: Scan bar code or enter JI number

**PROCEDURE NOTES**

A. Homocysteine increases in serum or plasma when separation from cells is delayed. This is due to the conversion of methionine to homocysteine by RBC.

**LIMITATIONS**

* 1. Evaluation of serum samples resulted in a median 9% increase in observed value compared to EDTA plasma samples.
  2. Patients taking methotrexate, nicotinic acid, theophylline, nitrous oxide, or L-dopa can have falsely elevated serum or plasma HCY levels
  3. S-adenosyl-methionine is an antidepressant that is structurally similar to S-adenosyl-homocysteine. Individuals taking this drug may show falsely elevated levels of HCVY, Refer to the Specificity section of the ADVIA Centaur HCY procedure for the cross-reactivity of S-adenosyl-methionine.
  4. It is not recommended that plasma and serum samples from the same patient be used interchangeably with this assay.
  5. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
  6. Interfering Substances:

|  |  |
| --- | --- |
| **Serum specimens that are...** | **Demonstrate < 5% change in results up to:** |
| Hemolyzed | up to 1.4% increase with 1000 mg/dL of hemoglobin |
| Lipemic | up to 2.5% increase with 1300 mg/dL of lipid |
| Icteric | up to 2.8% increase with 25 mg/dL of bilirubin |
| Proteinemic | up to 1.4 % increase with 6.5 g/dL of protein |

**REFERENCES**

A. ADVIA Centaur Assay HCY, 10629877\_EN Rev. H, 2011-07