# *Homocysteine*

ADVIA Centaur System

## *Principle of the Test*

The ADVIA Centaur® HCY assay is a competitive immunoassay using direct, chemiluminescent technology. The different forms of homocysteine in the patient sample are reduced to free HCY by the Reducing Reagent. Free homocysteine is then converted to S-adenosylhomocysteine (SAH) by the Enzyme Reagent. Converted SAH from the patient sample competes with SAH covalently coupled to paramagnetic particles in the Solid Phase for a limited amount of acridinium ester-labeled anti-SAH in the Lite Reagent.

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 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 in 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.

## *Clinical Application and Usefulness*

For *in vitro* diagnostic use in the quantitative determination of total homocysteine (HCY) in serum or EDTA plasma using the ADVIA Centaur System. This diagnostic test is designed to quantitatively measure HCY in serum or EDTA plasma. Such measurement can aid in the diagnosis and treatment of patients suspected of having homocysteinuria or hyperhomocysteinemia.

**WARNING**: Patients taking S-adenosyl-methionine may show falsely elevated levels of HCY. Patients taking methotrexate, nicotinic acid, theophylline, nitrous oxide, or L-dopa can have falsely elevated HCY levels.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this

interference and anomalous values may be observed. Additional information may be required for diagnosis.

# *Specimen Collection and Handling*

## Specimen Collection

**BIOHAZARD**
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Heparinized plasma is the recommended sample type for this assay. Serum is acceptable.
- **NOTE**: Do not use serum and heparinized plasma interchangeably from the same patient.
- This assay requires 20 µL of sample for a single determination. Additional volume is required for onboad dilutions.
- Centrifuge samples and remove serum or plasma from red blood cells as soon as possible after collection. Samples that cannot be separated soon after collection should be stored on ice until centrifugation. HCY increases in serum or plasma when separation from the cells is delayed.
- Allow serum samples to clot adequately before centrifugation.
- Samples are free of fibrin or other particulate matter.
- Samples are free of bubbles.

## Specimen Storage and Stability

- Do not store samples at room temperature.
- Samples are stored at 2 – 8˚C. Stable for up to 14 days refrigerated.
- Samples may be stored at or below -20°C for up to 13 weeks.
- Freeze samples only once and mix thoroughly after thawing.
- NOTE: centrifuge all samples prior to placing them on the analyzer. Spin samples for 7 minutes at 3,000 rpm in the IEC Centra-7 centrifuge.

## Specimen Rejection Criteria

Unlabelled specimens are rejected

Grossly hemolyzed samples are rejected

# Reagents

## Storage and Stability

- Store the reagents upright at 2–8 °C.
- Primary reagents stable until the expiration date on the pack label, or for 28 days onboard the system.
- Ancillary reagent and diluent stable until the expiration date on the pack label, or for 28 consecutive days after accessing the ancillary reagent pack.

**CAUTION:**

- Discard the primary reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

## Ingredients

Reagent ingredients for the ADVIA Centaur HCY assay are as follows:

| *Reagent* | *Volume* | *Ingredients* |
|---|---|---|
| Lite Reagent | 10.0 mL/ReadyPack® | monoclonal mouse anti-SAH antibody (~0.4 µg/mL) labeled with acridinium ester in phosphate buffer with bovine serum albumin and preservatives |
| Solid Phase | 25.0 mL/ReadyPack | SAH (~2.1 µg/mL) covalently coupled to paramagnetic particles in phosphate buffer with bovine serum albumin and preservatives |
| Enzyme Reagent (Primary reagent) | 5.0 mL/ReadyPack | bovine derived S-adenosylhomocysteine hydrolase enzyme (~45 mU/mL) in TRIS buffer with preservatives |
| Reducing Reagent (Ancillary reagent) | 10.0 mL/Ready Pack | dithiothreitol (~1.5 mg/mL) in citrate buffer with preservatives |
| HCY Diluent | 10.0 mL/ Ready Pack | phosphate buffer with bovine gamma globulin and preservatives |

**BIOHAZARD**
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

# Reagents Special Preparation

No special preparation of reagents is required.

## *Calibration*

For detailed procedural information about scheduling a calibration, refer to the ADVIA Centaur Reference Manual or to the online help system.

### Two-point Calibration Interval

Use HCY Calibrator to perform two-point calibrations.

Perform a two-point calibration every 14 days. Additionally, calibrate when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

### Defining Calibrator Values for Two-point Calibration

1. At the workspace, select **Calibration**.
2. Select **Calibrator Definition**.
3. Select **Scan Data**.
4. Scan the barcodes on the Calibrator Assigned Value Card. Scan from top to bottom using the "Centaur" labeled card.
5. Review the values on the Calibrator Assigned Value card to ensure that they are correct. Scan again, if required.
6. Select **Save**.

### Master Curve

Use the barcode reader to enter the Master Curve values from the Master Curve card onto the system. Ensure that the lot number on the Master Curve matches the lot number of the ReadyPack.

NOTE: the master curve card only needs to be scanned when a new lot of reagents are put into use.

### Defining the Master Curve Using the Barcode Scanner

1. At the workspace, select **Calibration**.
2. Select **Master Curve Definition**.
3. At the Calibration-Master Curve Definition window, select Scan Data.
4. Scan the barcodes on the HCY Master Curve Card. Scan from top to bottom using the "Centaur" labeled card.
5. Select **Save**.

# *Quality Control (QC)*

## QC Materials

BIORAD LIQUICHECK HOMOCYSTEINE CONTROL LEVELS 1 & 2

These controls are ready to use after being thawed and mixed well.

Storage: Controls are stored at -10°C to -20°C until the expiration date.
Stability: Once opened, the control is stable for 14 days at 4°C.

See posted QC chart for acceptability limits.

## QC Frequency

Analyze both levels of quality control material on each day that samples are analyzed.

Analyze both levels of quality control material each time a two-point calibration is performed.

## Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the HCY QC results do not fall within the defined ranges then the run is rejected, and you must take the following corrective action:

- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer Diagnostics
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary, recalibrate the system and then rerun the quality control samples or contact Bayer Diagnostics for more assistance

# *Instrument Operation and System Description*

The ADVIA Centaur system is an automated, random access, direct chemiluminescent immunoassay analyzer that offers no-pause reloading of reagents, samples, and supplies. Comprehensive assay groups are available.

When the sample start button is pressed, the barcode labels on the sample cups are read, sample is aspirated, reagent is dispensed, and the assay process begins. Paramagnetic particles are magnetically separated in the cuvette incubation ring. The addition of hydroxyl groups to complete the flash reaction is accomplished by the addition of Acid and Base. The chemiluminescent reaction occurs in the luminometer. The PMT measures the chemical light reaction that takes place.

Refer to Section 4 in the ADVIA Centaur Reference Manual for detailed procedures that describe how to schedule samples and manage the worklist.

## A. System Start Button

There is one (1) main system operation button on the ADVIA Centaur, the Start button. Pressing this button performs the following actions:

- Homes the subsystems.
- Starts specimen sampling.
- If the Start button is pressed while the system is processing samples, it stops sampling additional specimens, however it continues to process the specimens in the incubation ring.

## B. Start-up

1. Put the samples racks on the sample entry queue.
2. Press the Start button.

## C. Verify Supplies:

While the system is running, you can manage the following supplies without interrupting the run.

- Reagent water supply
- Liquid waste container
- Acid and Base reagents
- Cuvette waste bin
- Sample tip waste bin and tip tray waste area
- Cuvette supply
- Sample tip supply
- Ancillary reagent packs
- Primary reagent packs

## D. Scheduling a Run/Entering a Worklist

You can enter a worklist by different methods:

### *Automated Worklist*

You can send the worklist to the ADVIA Centaur from a Lab Information System (LIS). This is done in a unidirectional manner (Dynamic Download) or in a bi-directional manner (Host Query).

### *Manual (Operator-initiated) Worklist*

**Scheduling Calibrators**

1. At the workspace, select **Worklist** and then select **Schedule**.
2. Select the **Calibrator** box.
3. Select the test you want to schedule for calibration.
4. The reagent lot that is on the system and any defined calibrators are displayed. Select the appropriate reagent and calibrator lot combination.
5. Select **Save**.
6. Ensure that the lot numbers of reagent and calibrator are available for system use.

**Scheduling Controls**

1. At the workspace, select **Worklist** and then select **Schedule**.
2. Select the **Control** box.
3. Select each test you would like to schedule.
4. Select each control level and lot number you need to run.
5. Select **Save**.

**Scheduling Patient Samples**

1. At the workspace, select **Worklist** and then select **Schedule**.
2. Select the **Patient** box.
3. Enter a patient sample identification number (SID).
4. Press the **Enter** key.
5. Select each test, profile, dilution, and/or replicates needed.
6. Select **Save**.

For detailed operating procedural information, refer to the ADVIA Centaur Reference Manual or to the online help system.

## E. Loading Sample Racks

**BIOHAZARD**
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

**CAUTION:** Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Gently mix the calibrators (let them sit at room temperature for 5 minutes) and quality controls before dispensing into the sample cups.
2. Load the sample cups containing the calibrators, controls, or patient specimen into any Centaur rack.
3. Load racks onto the sample entry queue.
4. Press the Start button.

## F. Loading Ancillary Reagents

Place the ancillary reagent pack on the ancillary entry queue so that the barcode is facing out until the green light comes on. The ADVIA Centaur moves the pack into the ancillary queue.

## G. Loading Primary Reagents

**CAUTION:** Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing reagents for use, refer to Appendix C, *Handling Reagents,* in your ADVIA Centaur Assay Manual.

1. Gently mix the HCY ReadyPack before placing it on the reagent shelf for the first time.
2. Load the HCY ReadyPack by matching the color on the pack to the color-coded reagent shelf.
3. Close the reagent compartment door.

## H. Loading STAT Samples in the STAT Entry Queue

1. Press the Start button, if the green Start light is not lit.
2. Place the STAT sample tube or sample cup in the rack.
3. Load the rack in the STAT entry queue until the green STAT light comes on, indicating the system has accepted the rack.

## *Performance Characteristics*

### *Precision*

Seven samples were assayed 3 times for 20 days, 1 run per day, using a stored calibration. The total number of replicates was 60 for each sample. The following results were obtained:

| Mean HCY (µmol/L) | Within-run % CV | Run-to-run % CV | Total % CV |
|---|---|---|---|
| 4.9 | 4.4 | 5.2 | 6.8 |
| 7.9 | 4.2 | 2.6 | 4.9 |
| 9.9 | 3.7 | 2.9 | 4.7 |
| 22.2 | 3.0 | 1.5 | 5.4 |
| 26.4 | 2.5 | 2.5 | 3.5 |
| 43.0 | 2.6 | 4.0 | 4.7 |
| 61.6 | 2.3 | 3.1 | 3.9 |

### *Standardization*

The ADVIA Centaur HCY assay is traceable to an internal standard manufactured using highly purified material. Assigned values of calibrators are traceable to this standardization.

### *Specificity*

The ADVIA Centaur HCY assay shows high specificity for S-adenosyl-homocysteine, the enzymatically converted form of L-homocysteine. The following compounds were added at the concentration indicated to a sample with known HCY concentration. ADVIA Centaur HCY assay results from the spiked samples were compared with those of unspiked control samples.

Percent cross-reactivity is calculated as:

$$\% \text{ cross-reactivity} = \frac{(\text{concentration of spiked sample} - \text{concentration of unspiked sample}) \times 100}{\text{concentration of compound}}$$

| **Compound** | **Amount (µmol/L)** | **% Cross-Reactivity** |
|---|---|---|
| Adenosine | 900 | <0.01 |
| S-adenosyl-L-methionine | 80 | 6.6 |
| L-Cystathionine | 500 | 0.01 |
| L-Cysteine | 8000 | 0.03 |
| L-methionine | 800 | 0.21 |
| Glutathione | 500 | <0.01 |
| NAD | 1000 | 0.12 |
| Homocysteine Thiolactone | 50 | 1.3 |

### *Analytical Sensitivity*

The ADVIA Centaur HCY assay has an analytical sensitivity of ≤ 0.50 µmol/L. Analytical sensitivity is defined as the concentration of HCY that corresponds to the RLUs that are two standard deviations less than the mean RLUs of 30 replicate determinations of the HCY zero standard.

### *Analytical Measuring Range (AMR)*

The analytical measuring range of the ADVIA Centaur Homocysteine assay is 1 to 65 µmol/L.

## *Reporting Results*

### *Reportable Range*

The reportable range of the ADVIA Centaur HCY assay is 1 µmol/L to 650 µmol/L. Values below 1 µmol/L are reported as <1. Values above 650 umol/L are reported as >650.

### *Dilutions*

- **NOTE: samples with Homocysteine levels greater than 65 µmol/L are programmed to rerun on a 1:2 instrument performed autodilution.**
- **If upon a 1:2 autodilution the result is still above the AMR, then schedule a 1:10 autodilution on the Centaur. See dilution procedure**
- Patient samples can be automatically diluted by the system or prepared manually.
- **NOTE**: The sample volume required to perform onboard dilutions is as follows:

| *Dilution* | *Sample Volume (µL)* |
|---|---|
| 1:2 | 80 |
| 1:10 | 30 |

For automatic dilutions, ensure that ADVIA Centaur HCY Diluent is loaded on the Centaur.

For detailed information about automatic dilutions, refer to the system operating instructions or to the online help system.

- Use HCY Diluent to manually dilute patient samples, and then load the diluted sample in the sample rack, replacing the undiluted sample.
- Ensure that results are mathematically corrected for dilution. **If a dilution factor is entered when scheduling the test, the system automatically calculates the result.**

### *Reference Interval*

- less than or equal to 70 years:  4 to 14 umol/L
- greater than 70 years :  6 to 20 umol/L

### *Critical Values*

*Not applicable*

### *Reporting Protocol for Critical Values*

*Not applicable*

### *Units for Reporting Results*

The system reports serum HCY results in µmol/L. Units are user-defined in the system software.

## *Procedure Notes*

### *Calculations*

For detailed information about how the system calculates results, refer to the ADVIA Centaur Reference Manual or to the online help system.

### *Interchangeability of Sample Types*

Do not use serum and heparinized plasma interchangeably from the same patient.

### *Disposal*

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

## *Method Limitations*

Patients taking methotrexate, nicotinic acid, theophylline, nitrous oxide, or L-dopa can have falsely elevated serum or plasma HCY levels.

S-adenosyl-methionine is an antidepressant that is structurally similar to S-adenosyl-homocysteine. Individuals taking this drug may show falsely elevated levels of HCY.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

| *Serum specimens that are . . .* | *Demonstrate the following changes . . .* |
|---|---|
| hemolyzed | up to 1.4% increase with 10.0 g/dL of hemoglobin |
| lipemic | up to 2.5% increase with 1300 mg/dL of lipid |
| icteric | up to 2.8% increase with 25.0 mg/dL of bilirubin |
| proteinemic | up to 1.4% increase with 6.5 g/dL of protein |

**If there is an interferant (hemolysis, lipemia and icterus) higher than the values listed in the above table, run the sample undiluted and send the corresponding ETC Code:**

**TUR** "Specimen turbid, result may be invalid"

**ICTRQ** "Specimen icteric, result may be invalid"

**HEMRQ** "Specimen hemolyzed, result may be invalid"

For additional information on performance characteristics including cross reactivity and dilution recovery, see the product information in the ADVIA Centaur Assay Manual.

## *Equipment and Supplies*

- ADVIA Centaur HCY ReadyPack
- ADVIA Centaur HCY Reducing Reagent ReadyPack
- ADVIA Centaur HCY Calibrator
- ADVIA Centaur HCY Diluent
- ADVIA Centaur Sample Cups and Caps
- ADVIA Centaur Cuvettes
- ADVIA Centaur Tips
- ADVIA Centaur Cleaning Solution Concentrate
- ADVIA Centaur Acid Reagent (0.5% $H_20_2$, 0.1N $HNO_3$)
- ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
- Reagent Water

## References


1. Bayer Diagnostics ADVIA Centaur HCY product insert, Revision A.
2. Bayer Diagnostics ADVIA Centaur Reference Manual, Revision D.
3. Bayer Diagnostics ADVIA Centaur Assay Manual, Revision AT.
4. National Committee for Clinical Laboratory Standards (NCCLS). Clinical Laboratory Procedure Manuals—Third Edition (GP2-A3), 1996.


## Technical Assistance

Bayer Diagnostics Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number: IRL 80600727

Customer Account Number: 619504