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Diazyme HCY POC Test Kit

Configuration

The Diazyme Homocysteine (HCY) POC test kit is provided in the following kit configuration:

Instrument	Catalog No.	Kit size (20 tests)	
SMART 340 or SMART 700/340	DZ568B-SMA	DRS Cuvette (Reagent R1)	20 pcs
		DRS Cap (Reagent R2)	20 pcs
		RFID card	1 pc

* Diazyme Reagent System (DRS)

Intended Use

Diazyme's HCY POC Test Kit is intended to be used with the SMART analyzer in a Point-of-Care setting for the *in vitro* quantitative determination of total L-homocysteine in serum or plasma. The assay can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria. For *in vitro* diagnostic use only.

WARNING: 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-azuridine triacetate, may have higher levels of HCYdue to metabolic interference with homocysteine metabolism.

Clinical Significance

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 oxidized, protein bound and free).

Elevated levels of tHcy have emerged as an important risk factor in the assessment of cardiovascular disease and stroke [1-3]. Excess HCY in the bloodstream 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. Elevated tHcy levels are caused by four major factors, including: a) genetic deficiencies in enzymes involved in HCY metabolism, such as cystathionine beta-synthase (CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR); b) nutritional deficiency in B vitamins such as B_6, B_{12}, and folate; c) renal failure for effective amino acid clearance; and d) drug interactions that interfere with HCY metabolism, such as nitric oxide, methotrexate, and phenytoin.Elevated levels of tHcy are also linked with Alzheimer's disease[4] and Osteoporosis[5]. Guidelines for tHcy determination in clinical laboratories have recently been established[6].

Assay Principle

Diazyme HCY POC Test is based on a novel enzyme cycling method as published in the Journal of Clinical Chemistry[7]. In this assay, oxidized HCY is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM) catalyzed by a HCY S-methyltransferase to form methionin (Met) and S-adenosylhomocysteine (SAH). SAH is assessed by coupled enzyme reactions including SAH hydrolase, adenosine (Ado) deaminase and glutamate dehydrogenase, wherein SAH is hydrolyzed into adenosine (Ado) and HCY by SAH hydrolase. The formed HCY that is originated from the co-substrate SAM is cycled into the HCY conversion reaction by HCY S-methyltransferase. This forms a co-substrate conversion product-based enzyme cycling reaction system with significant amplification of detection signals. The formed Ado is immediately hydrolyzed into inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversion of NADH to NAD+. The concentration of HCY in the sample is indirectly proportional to the amount of NADH converted to NAD+ (ΔA340nm). The HCY concentration is expressed as µmol/L by use of a lot specific calibration curve that is stored in an RFID card provided with each SMART test kit.

Reagent Composition

Active Ingredients	Concentration
S-adenosylmethionine (SAM)	0.1 mM
NADH	>0.2 mM
TCEP	>0.5 mM
2-oxoglutarate	5.0 mM
Glutamate dehydrogenase	10 KU/L
SAH hydrolase	3.0 KU/L
Adenosine deaminase	5.0 KU/L
Hcy methyltransferase	5.0 KU/L

Materials Required but not Provided

SMART 340 (DZ90039) or SMART 340/700 (DZ90036) analyzer, HCY controls (DZ568A-CON), and sample rack (DZ90049).

Stability and Storage

The Diazyme HCY POC test kit should be stored at 2-8°C. **DO NOT FREEZE**. The reagents are stable when stored as instructed until the expiration date on the label. Do not mix reagents of different lots.

Specimen Collection and Handling

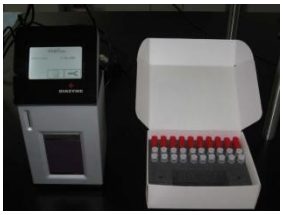
Fresh serum or plasma is the recommended sample for the HCY assay. Twenty microliters (20µl) of the plasma should be added to the provided DRS cuvette (Reagent 1) for HCY testing using a laboratory pipette (DZ90050). It is important to centrifuge blood samples immediately after blood collections. Hemolysed or turbid specimens are not recommended for HCY assay.

Assay Procedures

The step by step assay procedure is illustrated in the pictures listed below:

1. Power the SMART device and open the Diazyme Enzymatic HCY SMART Assay Kit box (Catalog number DZ568B-SMA) (picture 1 below).
2. Insert the provided RFID card (included in the kit box) into the SMART device (picture 2).
3. Take out one DRS cuvette and one DRS cap from the kit box, and set them on a sample rack (picture 3). **Note: The kit box should reside at room temperature for a minimum of 10 minutes before use.** Reagents are light sensitive, please close kit box lid immediately after removing needed reagents and return to 2-8C storage.
4. Add the 20 µl of sample to the DRS cuvette (Reagent 1) (picture 4).
5. Put the DRS Cap on the top of the DRS cuvette and snap the DRS cap into place (picture 5).
6. Press the first button from left side of the SMART device display screen to open the door. This is the measurement button . Input patient demographics by pressing the edit button ••• and then the confirm button ✓ when finished. (picture 6).
7. Insert the capped DRS cuvette into the cuvette holder hole on the door of the SMART analyzer (picture 7). To start the assay, close the door by pressing the confirm button ✓ on the screen.
   *Caution: Please carefully examine the capped DRS cuvette before inserting. If it is dirty, wipe the cuvette with clean tissue or similar material to ensure the cuvette surface is clean.
8. The result is displayed on the screen in approximately 13 min (picture 8).

1.



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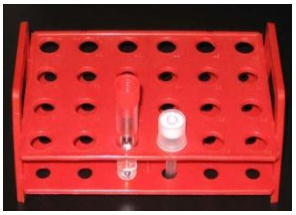

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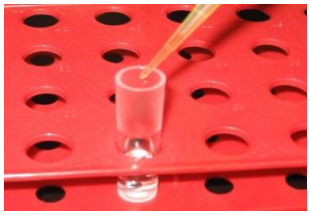

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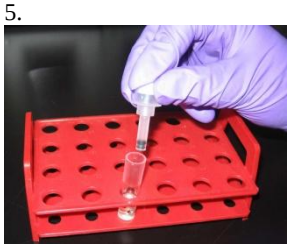

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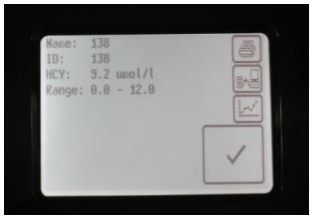

Precautions

1. Store the reagents at 2-8°C. Do not freeze the reagents.
2. Do not use the reagents after the expiration date labeled on the outer box.
3. DO NOT INGEST. Avoid contact with skin and eyes. Contains sodium azide which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of this reagent.
4. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
5. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.
6. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.

Calibration

The RFID card included with this kit contains calibration data. The calibration curve is stable until the printed expiration date. The user should put the RFID card (contains calibration data) in the instrument for every run.

Quality Control

We recommend that each laboratory use HCY controls to validate the performance of HCY reagents. A set of controls is available from Diazyme Laboratories (Cat. No. DZ568A-CON). The range of acceptable control limits should be established by individual laboratories.

Results

Results are displayed in µmol/L. Note: Samples with values greater than 50µmol/L should be diluted 1:1 with water and rerun. Multiply the results by 2.

Reference Range

In most of the U.S. clinical laboratories, 15µmol/L is used as the cut-off value for normal level of HCY for adults[10-12]. However, each laboratory is recommended to establish a range of normal values for the population in their region.

Limitations

- The measuring range of the assay is from 3.0 to 50.0 µmol/L. Samples with HCY values higher than 50.0µmol/L should be diluted 1:1 with water.
- Blood sample collectors that are claimed specifically for HCY assay cannot be used for the HCY POC Test as these sample collectors may contain SAH hydrolase inhibitor 3-deazaadenosine that interferes with enzyme cycling in the HCY assay.
- 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-azuridine triacetate may have higher levels of HCY due to metabolic interference with HCY metabolism.

Performance Characteristics

Precision

Within precisions for three levels of HCY serum samples are 3.2% for 7.5µmol/L Hcy, 1.8% for 11.87µmol/L Hcy, and 2.8% for 29.0 µmol/L Hcy. Total precisions (CV) for three levels of HCY serum samples are 3.4% for 7.5µmol/L Hcy, 3.5% for 11.87µmol/L Hcy, and 3.3% for 29.0µmol/L Hcy.

The assay precision was also evaluated at three physician office laboratories (POL) by intended users such as nurses and office assistances to test systemic and random error on three Diazyme HCY POC Test. A total of 5 serum samples containing HCY levels ranging from low to high were used for the precision study. At each site, 2 serum samples were tested. Each sample was run 4 times for 5 days.

The results are summarized in the following tables:

Sample	1	2	3	4	5
N	20	20	20	40	20
Mean	4.89	10.68	13.71	29.83	42.92
Within CV%	3.1%	2.8%	2.8%	3.5%	2.6%
Total CV%	5.2%	3.7%	4.1%	6.0%	3.2%

Additional precision was also evaluated at three physician office laboratories (POL) by intended users such as nurses and office assistances to test systemic and random error on three Diazyme HCY POC Test. A total of 9 different serum samples containing HCY levels ranging from low to high were used for the precision study. At each site, 3 serum samples were tested by three operators. Each sample was run 4 times for 5 days. The results are summarized in the following tables:

Site 1:

	Sample 1	Sample 2	Sample 3
No. of Points	20	20	20
Mean (µmol/L)	11.05	25.82	42.73
Within run CV	6.7%	6.2%	5.60%
Total CV	7.0%	5.3%	6.4%

Site 2:

	Sample 1	Sample 2	Sample 3
No. of Points	20	20	20
Mean (µmol/L)	10.26	25.18	41.99
Within run CV%	6.9%	5.2%	3.8%
Total CV%	6.6%	5.5%	4.4%

Site 3:

	Sample 1	Sample 2	Sample 3
No. of Points	20	20	20
Mean (µmol/L)	11.63	26.34	31.63
SD (µmol/L)	0.4941	1.9883	1.8298
Within run CV%	4.2%	7.5%	5.8%
Total CV%	6.0%	6.8%	5.5%

Limit of Quantitation

The LOB, LOD and LOQ of Diazyme Homocysteine POC Test Kit were determined according to CLSI EP17-A. LOB = 0.06 µmol/L; LOD = 0.32 µmol/L; LOQ = 3.0 µmol/L Homocysteine.

Linearity

Eleven levels of the Homocysteine linearity set were prepared by diluting a sample containing about 50.0 µmol/L Homocysteine with saline according to CLSI EP6-A and then were run with Diazyme Homocysteine POC Test Kit in triplicates. After linear regression, the correlation coefficient is $R^2 = 0.9992$, slope is 0.9749, and y intercept is 0.751. Diazyme Homocysteine POC Test Kit is linear up to 50.0 µmol/L. Analytical measuring range (AMR) is 3.0-50.0 µmol /L.

Interference

An interference study was performed by testing a serum sample spiked with varied concentrations of endogenous substances using Diazyme's HCY POC Test on the SMART Analyzer. The following substances normally present in the serum produced less than 10% deviation when tested at the stated concentrations: 40 mg/dL Bilirubin, 1000 mg/dL Triglyceride, 500 mg/dL Hemoglobin, 40 mg/dL Bilirubin Conjugate, and 10 mM Ascorbic Acid.

The following substances produced less than 10% deviation when tested at levels equal to the concentrations listed below:

Interference	Concentration
Glutathione	500 µM
Methionine	20µM
Cysteine	1000µM
Pyruvate	500µM
Cystathionine	100µM
Hydroxylamine	1000µM
Carbamezapine	130µM
Methotrexate	2.0mM
Phenytoin	200µM
6-azauridine triacetate	1000µM
S-adenosyl-methionine	20µM
Carbamezapine-10, 11-epoxide	60µM
Ethosuximide	1800µM
Primidone	200µM
Valporic Acid	3.5mM
Sodium Nitrate	500µM

Method Comparison

Correlation studies were done by testing 74 human serum samples on the SMART Analyzer and running the same samples in parallel on the Olympus AU400 using commercially available assay. The regression results are summarized in the following table:

n	74
Slope	0.9612
Intercept	0.5246

Correlation coefficient	0.9696
Range of values	4.17-49.50 µmol/L

The method comparison study was performed externally at the three POL sites. One Hundred and Twenty (120) human serum specimens are tested in total (40 samples at each site) on with the Diazyme Homocysteine POC reagents on SMART analyzers and with a predicate device on Olympus AU400. Regression analysis of the results obtained from the three POL sites is summarized as follows:

	Site 1	Site 2	Site 3	All 3 sites
N	40	40	40	120
Slope	1.0890	1.0041	1.0600	1.0552
Intercept	-0.7438	-0.6251	-1.1564	-0.8860
R^2	0.9830	0.9645	0.9819	0.9765
Range	5.43-48.95	3.88-45.43	4.81-49.86	3.88-49.86

References

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