SMART HbA1c Assay

Configuration

The SMART HbA1c Assay kit and SMART Analyzer is provided in the indicated packaging configuration:

Kit Catalog No.:	DZ168B-POC	(20 tests)
	ST0110	(24 tests)
Kit Components:	Lysis buffer	Pre-filled tubes
	(R1a): DRS Cuvette	Single use cuvette
	R1b bottle	1 brown bottle
	(R2): DRS Cap	Single use caps
	RFID card	Lot specific
	Capillary tubes	Disposable
Instrument:	Catalog No. (DZ90037)	SMART 700
	Catalog No. (DZ90036)	SMART 700/340

DRS: Diazyme reagent system.

Intended Use

SMART HbA1c Hemoglobin A1c (glycated hemoglobin A1c; A1c; HbA1c) reagents are intended for use with the SMART analyzer for the quantitative determination of stable HbA1c in human capillary and venous whole blood samples. Measurement of hemoglobin A1c is a valuable indicator for long-term diabetic control. For *in vitro* diagnostic use only.

Clinical Significance

Hemoglobin A1c is an important test recommended by the American Diabetes Association (ADA) and its usefulness was clarified by the United Kingdom Prospective Diabetes Study (UKPDS) and Diabetes Control and Complications Trial (DCCT). Currently, the HbA1c test is recommended for patients with diabetes every 2-3 months as part of the patient Diabetes management program. Glycohemoglobin is produced by non-enzymatic addition of glucose to amino groups in hemoglobin. HbA1c refers to glucose modified hemoglobin A (HbA) specifically at N-terminal valine residues of hemoglobin beta chains. HbA1c test is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications.[1-3] Therefore, the HbA1c test is a good indicator of glycemic control in the preceding 2-3 months.

Assay Principle

The SMART HbA1c test is an enzymatic assay in which lysed whole blood samples are subjected to extensive protease digestion with Bacillus *sp* protease. This process releases amino acids including glycated valines from the hemoglobin beta chains. Glycated valines then serve as substrates for specific recombinant fructosyl valine oxidase (FVO) enzyme, produced in *E. coli*. The recombinant FVO specifically cleaves N-terminal valines and produces hydrogen peroxide. This, in turn, is measured using a horseradish peroxidase (POD) catalyzed reaction and a suitable chromagen. The HbA1c concentration is expressed directly as %HbA1c by use of a lot specific calibration curve that is stored in an RFID card provided with each SMART test kit.

Reagent Composition

Component	Concentration
Lysis Buffer	
CHES, pH 8.7	100 mM
Triton-X-100	1 %
SDS	0.45 %
Redox Agents	0.5 mM
Reagent R1a	
MES pH 7.0	5 mM
Proteases	4 KU/mL
Triton-X-100	0.5%
Redox agents	>10 µM
Reagent R1b	
MES pH 6.3	1 mM
Redox agent	<7 mM
Triton-X-100	0.1 %
Reagent R2	
Tris pH 8.0	15 mM
FVO enzyme	>10 U/mL
POD	90 U/mL
Chromagen	0.8 mM

Materials Required but not Provided

SMART 700nm analyzer (DZ90037) or SMART 700/340nm dual wavelength analyzer (DZ90036), fixed volume transfer pipette 100 µL (DZ90091), bi-level SMART HbA1c controls (DZ168B-CON), optional sample rack (DZ90048). Note that disposable single-use lancets are also not provided.

Reagent Stability and Storage

Reagents are stable until their expiration date when stored at 2-8°C. **Reagents are light sensitive.**

Specimen Collection and Handling

The assay is formulated for use with human whole blood samples. Both finger prick capillary whole blood and venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 2 weeks of collection when stored refrigerated.[4] Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes. Note: Human specimens and all materials that are in contact with samples should be handled and disposed of according to local and national laws and as if such samples are capable of transmitting infection.

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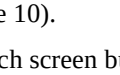
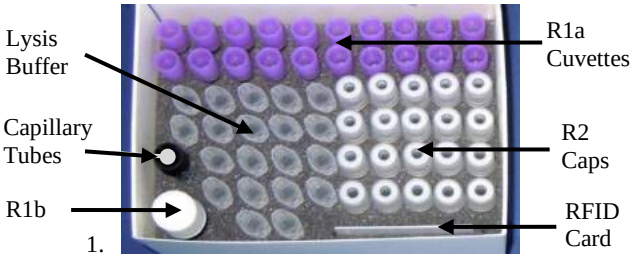
Assay Procedure

The step by step assay procedure is described below and illustrated in the accompanying pictures.

1. Power on SMART analyzer. The SMART analyzer is designed to be left powered on and does not need to be turned off after each use.
2. Examine Kit contents (picture 1). Insert the provided lot specific RFID test card into the SMART analyzer (picture 2) for each run. RFID card contains a preprogrammed lot specific calibration curve for the assay.
3. Determine the number of tests to be performed. For example, for a single test, take one lysis buffer (prefilled) micro tube, one DRS Cuvette, one DRS Cap and R1b bottle from the kit box and place on a kit rack (picture 3). Equilibrate at room temperature for a minimum of 10 minutes before use. Reagents are light sensitive.

For finger prick blood samples, go to step 4. For venous blood samples, use capillary tube provided to aspirate blood from primary tube until it reaches to the end and then go to step 6.

4. Prick patient finger with a disposable single-use lancet (not provided) (picture 4).
5. Use capillary tube provided to aspirate blood from the patient's finger until it reaches to the end (picture 5).
6. Drop the end-to-end filled capillary tube into micro tube prefilled with lysis buffer (picture 6) and invert sharply 3-4 times and then shake the tube vigorously 30 times (or 15 seconds) to prepare blood lysate**. (Make sure that the blood comes out of the capillary tube completely and is dispersed in lysis solution.)**
7. Place the micro tube containing the blood lysate on the kit rack and wait for 10 min (picture 7).
8. Using 100 µL fixed volume pipette with appropriate tip transfer 100 µl of R1b from brown glass bottle (picture 8a) into the DRS Cuvette (Reagent R1a) (picture 8b). Dispose the tip after use.
9. Using the 100 µL fixed volume pipette with a new tip, transfer 100 µl of the blood lysate from the micro tube from step 7 into the DRS Cuvette from step 8. **Make sure there are no bubbles in the tip.** (Picture 9a, 9b).
10. Place DRS Cap on top of the DRS Cuvette and snap into place (picture 10).
11. Press the touch screen button () located on lower left side of display screen to open the door (picture 11).
12. Insert the capped DRS Cuvette into the cuvette holder of the SMART analyzer door (picture 12). Do not push the door by hand. To start the assay, close the door by pressing the check button (✓) on the screen.
13. Assay result will be displayed on the screen in approximately 7 min (picture 13).



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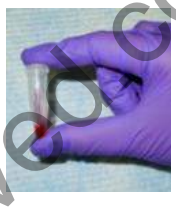
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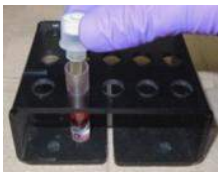
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Precautions

1) As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
2) Do not use the reagents past expiration date labeled on the outer box.
3) Do not mix reagents or RFID cards from different lots. RFID card contains lot specific calibration information only and is used with every run.
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). Dispose biohazard materials according to local, state and federal regulations in your country.
5) Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
6) 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.

Quality Control

SMART HbA1c assay control set (DZ168B-CON) can be purchased separately. Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls and handling of bio-hazardous material. To ensure adequate quality control, level 1 and level 2 controls with known values should be run as unknown samples.

Results

The HbA1c concentration is expressed directly as %HbA1c. The values reported are aligned with the Diabetes Control and Clinical Trials (DCCT) system and hence reported in the NGSP[6] format. No calculation step is needed.

The International Federation of Clinical Chemistry (IFCC) values (mmol/mol) can be calculated by use of the published[6, 7, 8] conversion formula: NGSP = [0.09148 x (IFCC)] + 2.152.

Reference Range

Non-diabetic individuals have HbA1c values in the range of 4-6% using a DCCT based assay.[5] The American Diabetes Association (ADA) recommends that the primary treatment goal in diabetes should be glycemic control equal to that achieved during the DCCT.[5] ADA states that lowering HbA1c to below or around 7% has been shown to reduce microvascular and neuropathic complications of type 1 and type 2 diabetes. Therefore, for microvascular disease prevention, the HbA1c goal for nonpregnant adults in general is <7% HbA1c.[5] However, each laboratory must establish its own normal range in their country of business taking into account sex, age and ethnicity. ADA recommends that glycemic control goals should be individualized based on duration of diabetes, age/life expectancy, comorbid conditions, known CVD or advanced microvascular complications, hypoglycemia unawareness and individual patient considerations.[5] More or less stringent glycemic goals may be appropriate for individual patients.[5]

Limitations

- The linearity of the assay is up to 12% HbA1c. Samples with values above 12% **should not be diluted** and retested. Instead the values should be reported as higher than 12% (>12%).
- The assay is formulated for use with human whole blood samples. Total hemoglobin in the sample should be in the range: 9-21 g/dL
- High HbF (>10%) may result in inaccurate HbA1c values.

Performance (SMART Analyzer)

Accuracy

The following HbA1c value data were obtained by comparing SMART HbA1c Assay to a legally marketed HbA1c Assay method on Hitachi 917 testing venous whole blood samples.

Venous blood comparison with predicate	Whole blood application
n	64
Slope	0.96
Intercept	0.30
Correlation coefficient	0.94
Range of values	4.5% - 11.1% HbA1c

Precision

The precision of the SMART HbA1c Assay was evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline with the following modifications: in the study, two whole blood specimens containing 5.6% and 7.4% HbA1c and one whole blood based HbA1c control containing 11.5% HbA1c were tested 2 runs per day in duplicates over 10 working days. The precision evaluation sample data is listed below:

Within Run precision CV%

	5.6% HbA1c	7.4% HbA1c	11.5% HbA1c
Total data points	40	40	40
Mean (%)	5.4	7.4	11.4
SD (%)	0.13	0.22	0.13
CV%	2.4%	3.0%	1.2%

Total Precision CV%

	5.6% HbA1c	7.4% HbA1c	11.5% HbA1c
Total data points	40	40	40
Mean (%)	5.4	7.4	11.4
SD (%)	0.15	0.19	0.15
CV%	2.70%	2.50%	1.30%

Linearity

SMART HbA1c Assay has a linear range from 4.0% - 12.0%.

Interference

The following interfering substances at the indicated concentrations produce less than 10% deviation when tested at indicated concentrations: ascorbic acid 30 mg/dL, total bilirubin 15 mg/dL, bilirubin (conjugated) 5 mg/dL, glucose 5000 mg/dL, triglyceride 4000 mg/dl, uric acid 30 mg/dL, and urea 100 mg/dL. Stable glycated hemoglobin serves as a substrate for enzymatic reaction used in the SMART HbA1c Assay. Carbamylated and labile HbA1c do not adversely affect the enzymatic reaction used in this assay. Variant hemoglobin AD, AE, AS, AC, and AF variants do not significantly interfere with the SMART HbA1c Assay.

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References

1. Goldstein, D.E. *et al*, Diabetes Care, 27(7):1761-73 (2004)
2. United Kingdom Prospective study, Lancet. 352: 837-53 (1998)
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4. Little, R. *et al.*, Clin Chemistry, 47: 1985-1992 (2001)
5. American Diabetes Association Clinical Practice Recommendations: Executive Summary: Standards of Medical Care in Diabetes - 2009 Diabetes Care, 32, suppl.1: S6-7 (2009)
6. NGSP, http://www.ngsp.org/ngsp.html
7. Hoelzel W *et al*. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan and Sweden: a method-comparison study. Clin Chem 2004;50:166-74
8. Sacks, D (ed). Global Harmonization of Hemoglobin A1c. Clin Chem 51(4): 681-683 (2005)

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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.

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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).

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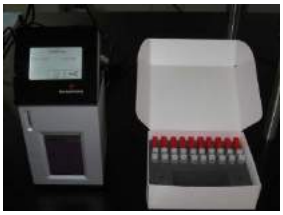

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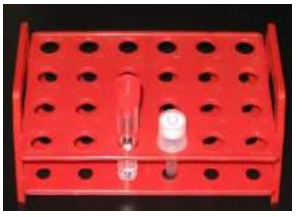

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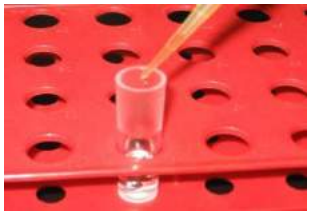

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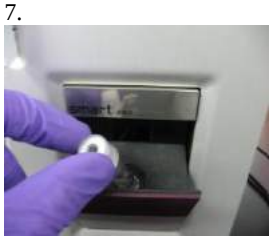

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Precautions

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.

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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

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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

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Diazyme hsCRP POC Test Kit

Configuration

The Diazyme hsCRP POC Test Kit is provided in the following kit configurations:

Instrument	REF	Kit size	
SMART 700	DZ135B-SMA	40 Test	
		DRS* Cuvette (Reagent 1)	40 pcs
		DRS* Cap (Reagent 2)	40 pcs
		RFID card	1 pc

* DRS: Diazyme Reagent System (DRS)

Intended Use

The Diazyme hsCRP POC Test Kit is for the *in vitro* quantitative determination of C-reactive protein (CRP) in human venous whole blood on SMART analyzers. Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. For *in vitro* diagnostic use only.

Clinical Significance [1-7]

CRP (C-reactive protein) is an acute phase protein whose concentration is seen to increase as a result of the inflammatory process, most notably in response to pneumococcal (bacterial) infectious, histolytic disease and a variety of disease states. Originally discovered by Tillet et al. in 1930 in patient sera with acute infection, CRP has now come to be used as a marker or general diagnostic indicator of infections and inflammation, in addition to serving as a monitor of patient response to therapy and surgery. Published literature states that regular measurements of CRP in infants can be a useful aid in the early diagnosis of infectious disease.

Assay Principle

Diazyme hsCRP POC Test Kit assay is based on a latex enhanced immunoturbidimetric assay on Diazyme's SMART analyzer. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP which has been sensitized to latex particles, agglutination results. This agglutination is detected as an absorbance change (700 nm), with the magnitude of the change being proportional to the quantity of CRP in the sample. The instrument calculates the CRP concentration of patient specimen by use of a lot specific calibration curve that is stored in an RFID card provided with each hsCRP POC kit. The RFID card is inserted in the SMART analyzer and is required for every sample tested.

Reagent Composition

Reagent	Composition
REAGENT 1	100 mM Tris-buffer solution, ready to use
REAGENT 2	Suspension of anti-human CRP coated latex particles (< 0.5%), ready to use

Precautions

1. 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).
2. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
3. Do not use the reagents after the expiration date labeled on the outer box.
4. 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.
5. 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.
6. Each donor unit used in the preparation of this product was tested by FDA-approved methods for the presence of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2), as well as for Hepatitis B surface Antigen and antibody to Hepatitis C Virus (HCV), and found to be negative (not repeatedly reactive). Because no testing can offer complete assurance that these or other infectious agents are absent, this material should be handled using good laboratory practice to avoid skin contact and ingestion.

Reagent Handling

1. The Diazyme hsCRP POC REAGENT are provided ready to use.
2. Physiological saline is needed to dilute high CRP samples.

Reagent Stability and Storage

Diazyme hsCRP POC Test Kit REAGENT and CONTROL materials should be stored at 2-8°C. **DO NOT FREEZE**. The REAGENT and CONTROL are stable when stored as instructed until the expiration date on the label. Do not mix REAGENT materials of different lots.

Specimen Collection and Handling

Venous Whole Blood EDTA samples are used for the assay. Collect venous whole blood by venipuncture. After collection, the whole blood sample is stable for at least 3 days when stored at 2-8°C. Prior to testing gently mix sample thoroughly.

Note: Human specimens and all materials that are in contact with samples should be handled and disposed of according to local and national laws and as if such samples are capable of transmitting infection

Materials Provided

Please refer to the table in the "Configuration" Section.

Materials Required but not Provided

SMART 700nm analyzer (REF DZ90037) or SMART 700/340nm dual wavelength analyzer (REF DZ90036), fixed volume transfer pipette 20 µL (REF DZ90092) Controls for validating the performance of the hsCRP POC assay kits are provided separately (REF DZ135B-CON), optional sample rack (REF DZ90048).

Assay Procedures

The step by step assay procedure is illustrated below:

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1.

2.

3.

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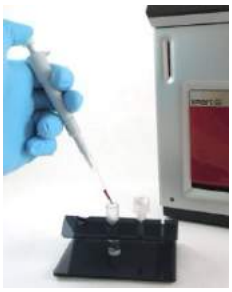

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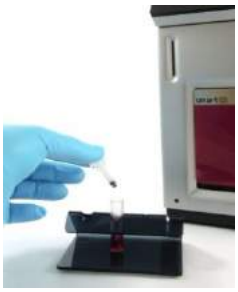

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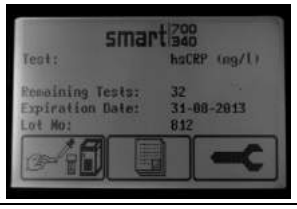

7.

8.

9.

Assay procedure:

1. Power on the SMART device and open the Diazyme hsCRP POC Test Kit box (REF: DZ135B-SMA) (image 1).
2. Insert the provided RFID card (included in the kit box) into the SMART device (image 2).
3. Take out one DRS cuvette and one DRS cap from the kit box, and set them on a sample rack (image 3).

   Note: The kit box should equilibrate at room temperature for a minimum of 10 minutes to allow material to warm up to room temperature before use.
4. Add 20 µl of sample into the DRS cuvette (Reagent 1) (image 4).
5. Place the DRS Cap on the top of the DRS cuvette and snap the DRS cap into place (image 5).
6. Press the first button on the far left side of the SMART device display screen to open the door (via Measurement touch screen button). Input patient demographics by pressing the Edit button and then press the confirm button when finished (image 6).
7. Insert the capped DRS cuvette into the cuvette holder on the door of the SMART analyzer (image 7).

   Caution: Carefully examine the capped DRS cuvette before inserting into analyzer. If the cuvette is dirty, wipe the cuvette with a clean tissue or similar non abrasive cloth to ensure the cuvette surface is clean.
8. To start the assay, close the analyzer door by pressing the Confirm button on the screen (image 8). **Note: Do not manually push the analyzer door close by hand use the touch screen button only.**

   The result is displayed on the analyzer touch screen in approximately 4 min (image 9).

Quality Control

We recommend that each laboratory use hsCRP CONTROL to validate the performance of CRP REAGENT. A set of normal and abnormal ranges of hsCRP CONTROL material is available from Diazyme Laboratories (REF DZ135B-CON). The range of acceptable control limits should be established by individual laboratories.

Each laboratory should follow federal, state, and local guidelines for quality control.

Results

Results are printed out in mg/L. Note: Samples with CRP value above 26mg/L should be diluted 1:1 with saline and rerun and multiply obtained value by 2.

Reference Range

The assay reference interval was determined using whole blood specimens from 150 apparently healthy adults with age of 19-63 according to CLSI C28-A3 guideline. The whole blood specimens were tested in duplicate by the Diazyme hsCRP POC Test Kit. EP Evaluator 8 Software was used to verify the reference interval. The results showed that < 5.0 mg/L CRP was obtained in 95% of the population tested. Results are based on a non parametric approach.

However, each laboratory is recommended to establish a range of normal values for the population in their region.

Limitations

1. A sample with a CRP level exceeding the linearity limit of 26 mg/L should be diluted with 0.9% saline and re-assayed incorporating the dilution factor in the calculation of the value.
2. Store the reagents at 2-8°C. **Do not freeze the REAGENT.**

Analytical Characteristics

Analytical characteristics were determined by Diazyme Laboratories using automated procedures on SMART analyzers. Forty one (n = 41) serum samples ranging from 0.47 - 24.97 mg/L were paired with whole blood samples gave a correlation coefficient of 0.9703. Linear regression analysis gave the following equation: This whole blood method yields the following equation:

y = 0.9871 (reference method) - 0.4005 mg/L

Method comparison was also performed at three (3) POL sites by intended users. One hundred and twenty (n = 120) paired human whole blood-serum EDTA samples were tested for comparison. The regression results are summarized in the following table:

	Site 1	Site 2	Site 3	Mean of Sites
n	40	40	40	120
Slope (w/ 95% Confidence Interval)	0.9515 (0.8996 - 0.9927)	1.0108 (0.9508 - 1.0484)	1.0106 (0.9017 - 1.0369)	0.9874 (0.9420 - 1.0056)
Intercept (w/ 95% Confidence Interval)	-0.0326 (-0.0395 - 0.1689)	0.0556 (-0.1744 - 0.1349)	-0.2896 (-0.3237 - 0.0040)	-0.1282 (-0.0463 - 0.0686)
Correlation coefficient	0.9897	0.9918	0.9902	0.9890
Range of values	0.60-25.02	0.47-25.05	0.51-25.89	0.47-25.89

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Precision

The assay precision was evaluated by testing the two level specimens (low = 0.80 mg/L, 3.25 mg/L, and high = 12.5 mg/L) in 4 runs per day for 10 days on SMART analyzers. Precision data is summarized in the table below:

Within Run precision

	Sample 1 0.80 mg/L	**Sample 2** 3.25 mg/L	**Sample 3** 12.50 mg/L
n	40	40	40
Mean (mg/L)	0.813	3.186	12.560
SD	0.0782	0.0901	0.2247
CV	9.62%	2.83%	1.79%

Total Precision

	Sample 1 0.80 mg/L	**Sample 2** 3.25 mg/L	**Sample 3** 12.50 mg/L
n	40	40	40
Mean (mg/L)	0.813	3.186	12.560
SD	0.0664	0.0984	0.2667
CV	8.17%	3.09%	2.12%

The precision was also evaluated at three (3) physician office laboratories (POL) by intended users such as nurses and office assistants. Six (n = 6) whole blood samples containing CRP levels ranging from low to high were used for the external precision study. At each site, 2 whole blood samples were tested. Each sample was run 4 times per day for 5 days using three SMART Analyzers.

Within Run precision

	Site 1		**Site 2**		**Site 3**	
	1	2	3	4	5	6
n	20	20	20	20	20	20
Mean (mg/L)	0.798	4.796	0.758	17.796	7.780	18.725
SD	0.0372	0.3369	0.0684	0.7447	0.4225	0.9409
CV	4.67%	7.02%	9.04%	4.18%	5.43%	5.02%

Total precision

	Site 1		**Site 2**		**Site 3**	
	1	2	3	4	5	6
n	20	20	20	20	20	20
Mean (mg/L)	0.798	4.796	0.758	17.796	7.780	18.752
SD	0.0684	0.3674	0.0616	0.7153	0.4082	0.8951
CV	8.58%	7.37%	8.13%	4.02%	5.24%	4.78%

Linearity

Linearity studies using SMART analyzers showed that Diazyme hs-CRP POC Test Kit has a linear range from 0.47 to 28.00 mg/L.

Dilution	**Mean Recovery (mg/L)**	**Expected Recovery (mg/L)**	**Error (mg/L)**	**% Error**
Level 0	0.51	0.47	-0.09	-9.2%
Level 1	0.72	0.75	0.04	3.6%
Level 2	1.24	1.25	0.01	1.1%
Level 3	3.96	4.00	0.01	0.9%
Level 4	10.15	10.00	-0.02	-1.5%
Level 5	13.06	13.00	0.00	-0.5%
Level 6	16.06	16.00	0.00	-0.4%
Level 7	18.94	19.00	0.00	0.3%
Level 8	22.31	22.00	-0.01	-1.4%
Level 9	25.15	25.00	-0.01	-0.6%
Level 10	28.02	28.00	0.00	-0.1%

Interference

Assay is not affected by conjugated bilirubin up to 30 mg/dL, unconjugated bilirubin up to 40 mg/dL, triglycerides up to 1000 mg/dL, hemoglobin up to 20 g/dL, Rheumatoid Factor up to 250 IU/mL, ascorbic acid up to 176 mg/dL, oxaloacetate up to 200 μM, glutathione up to 200 μM, isoniazid up to 200 μM, and L-DOPA up to 200 μM. Higher levels may cause interference.

LOB, LOD, and LOQ

The LOB, LOD, LOQ of the Diazyme hsCRP POC Test Kit was determined according to CLSI EP17-A. By testing a True Blank Sample (7.5% BSA) in 20 replicates daily for 3 days, LOB was determined to be 0.055 mg/L; By testing five low serum samples (100 time diluted) in 4 replicates for 3 days, LOD was determined to be 0.15 mg/L; To determine LOQ, specimens with mean measured concentrations ranging from 1.50 to 0.12 mg/L were assayed. Based on the EP evaluator-8 fitted model, the LOQ (lowest concentration for which CV is less than a target of 20% with 95% of confidence interval) is 0.47 mg/L CRP

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Diazyme Cystatin C POC Test Kit

Configuration

The Diazyme Cystatin C POC Test Kit is provided in the following kit configurations:

Instrument	Kit Catalog #	Kit size	
SMART 700 or SMART 700/340	DZ133D-SMA	DRS* Cuvette (Reagent R1)	20 pcs
		DRS Cap (Reagent R2)	20 pcs
		RFID card	1 pc

* DRS: Diazyme Reagent System (DRS)

Intended Use

Diazyme Cystatin C Point-of Care (POC) test reagents are intended for use with the SMART analyzer for the quantitative determination of Cystatin C in venous whole blood by latex enhanced immunoturbidimetric method. The measurement Cystatin C is used as an aid in the diagnosis and treatment of renal disease. For *in vitro* Diagnostic Use Only.

Clinical Significance[1-7]

Cystatin C is a basic proteinase inhibitor with a low molecular mass of 13Kda that is produced at a constant rate in all nucleated cells and appears in human plasma and serum. Cystatin C is freely filtered through the glomerulus, is not secreted by the tubule or eliminated via any extra-renal route, and is almost completely absorbed and catabolized by proximal tubular cells. Therefore, the plasma concentration of Cystatin C is almost exclusively determined by the glomerular filtration rate (GFR), making Cystatin C an excellent indicator of GFR.

Assay Principle

Diazyme's Cystatin C POC Test Kit is based on a latex enhanced immunoturbidimetric assay on the SMART analyzer. The whole blood is lysed upon mixing with reagent R1. Cystatin C in the sample binds to the specific anti-Cystatin C antibody, which is coated on latex particles, and causes agglutination. The degree of turbidity caused by agglutination can be measured optically and is proportional to the amount of Cystatin C in the sample. The instrument calculates the Cystatin C concentration of patient whole blood specimens by use of a lot specific calibration curve, in which Cystatin C levels are normalized to serum/plasma levels. The curve is represented in a Calibration card (RFID) provided with each Cystatin C POC Test Kit.

Reagent Composition

Reagent 1

Tris-buffer solution, ready to use

Reagent 2

Suspension of anti-human Cystatin C chicken polyclonal antibodies coated latex particles, ready to use

Materials Required but not Provided

SMART 700nm Analyzer (DZ90037) or SMART 340/700nm Analyzer (DZ90036), Controls for validating the performance of the Cystatin C reagents are provided separately (DZ133D-CON).

Reagent Stability and Storage

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

Specimen Collection and Handling

EDTA whole blood samples can be used for the Cystatin C POC test. Samples should be analyzed within 3 days if stored at 2-8°C.

Note: Human specimens and all materials that are in contact with samples should be handled and disposed of according to local and national laws and as if such samples are capable of transmitting infection.

Assay Procedures

The step by step assay procedure is illustrated below:

1. Power the SMART device and open the Diazyme Cystatin C POC Test Kit box (Cat. No. DZ133D-SMA) (image 1).
2. Insert the provided RFID card (included in kit box) into the SMART device (image 2).
3. Take out one DRS cuvette and one DRS cap from the kit box, and set them on a sample rack (Image 3).

   Note: The kit box should equilibirate at room temperature for a minimum of 10 minutes to allow material warm up to room temperature before use.
4. Add 20µl of sample into the DRS cuvette (Reagent 1) (image 4).
5. Place the DRS Cap on the top of the DRS cuvette and snap the DRS cap into place (image 5).

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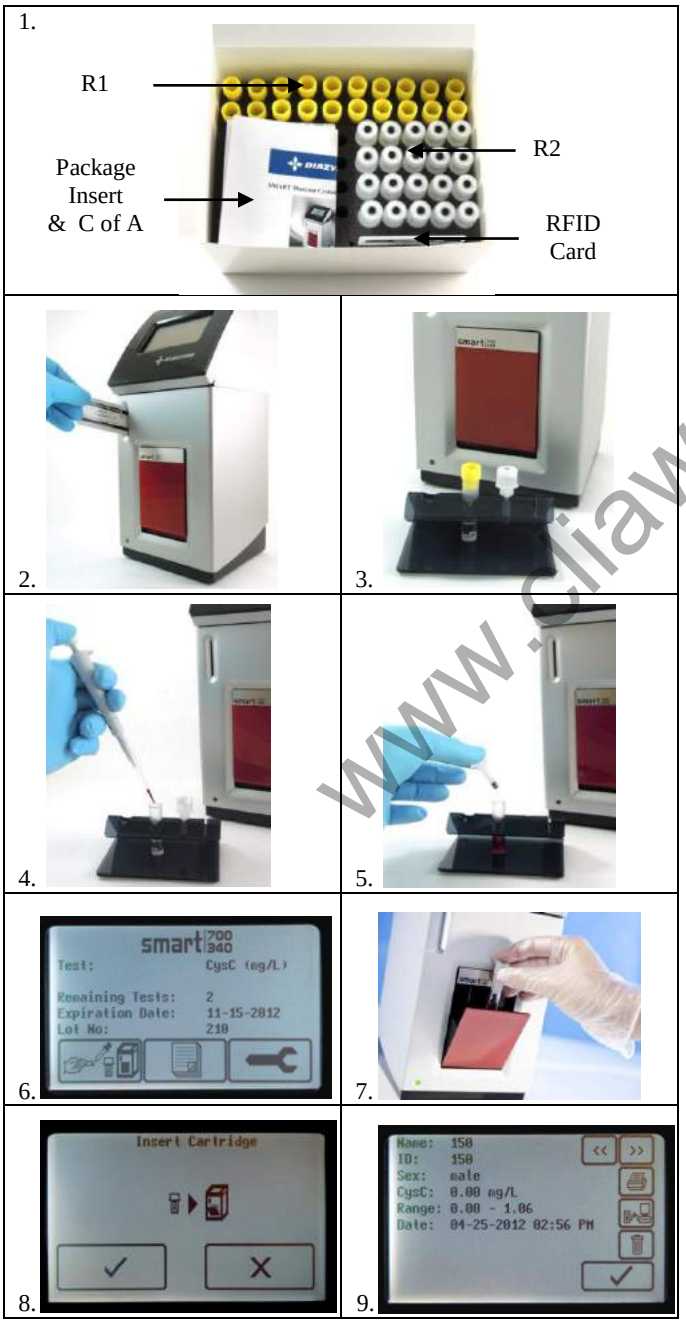
6. Press the first button on the far left side of the SMART device display screen to open the door (via Measurement touch screen button). Input patient demographics by pressing the Edit button and then press the confirm button when finished (image 6).
7. Insert the capped DRS cuvette into the cuvette holder on the door of the SMART analyzer (image 7).

   Caution: Carefully examine the capped DRS cuvette before inserting into analyzer. If the cuvette is dirty, wipe the cuvette with a clean tissue or similar non abrasive cloth to ensure the cuvette surface is clean.
8. To start the assay, close the analyzer door by pressing the Confirm button on the screen (image 8). **Note: Do not manually push the analyzer door close by hand use the touch screen button only.**
9. The result is displayed on the analyzer touch screen in approximately 10 min (image 9).



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.

Quality Control

Diazyme Cystatin C POC Control set can be purchased separately (Cat. No. DZ133D-CON).

The Cystatin C POC controls can be used to validate the performance of Cystatin C reagent kit. A set of normal and abnormal ranges of SMART Cystatin C controls is available from Diazyme Laboratories (Cat. No. DZ133D-CON). The range of acceptable control limits should be established by individual laboratories.

To ensure adequate quality control test Diazyme Cystatin C POC Control set weekly and when a changing to a new lot of reagent kit. Controls are treated exactly the same as samples when following assay procedure.

Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls and handling of bio-hazardous material.

Results

Results are printed out in mg/L. Note: Samples with values greater than 7.65 mg/L should be diluted 1:1 with saline and rerun. Multiply results by 2.

Reference Range

The assay reference interval was determined using human whole blood specimens from 126 apparently healthy adults with age of 19-63 according to CLSI C28-A3 guideline. EP Evaluator 8 Software was used to establish the reference interval. The reference range was established to be 0.46-1.06 mg/L, which is similar to the published range of 0.50 - 1.03 mg/L[6]. However, each laboratory is recommended to establish a range of normal values for the population in their region.

Limitations

A sample with a Cystatin C level exceeding the linearity limit of 7.65 mg/L should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

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Performance Characteristics

Precision

The precision of the Diazyme Cystatin C POC Test Kit was evaluated according to modified CLSI EP5-A guideline. The CV for samples above 1.0 mg/L ranged from 2.2% to 4.9%. Samples with concentrations of 0.70 mg/L, and 0.99 mg/L were also tested and the CV ranged from 6.9% to 5.6%.

The 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 Cys C SMART assay. At each site, 4 whole blood samples were tested. Each sample was run 4 times per day for 5 days. A total of 6 whole blood samples containing Cystatin C levels ranging from low to high were used for the precision study. The CV for samples above 1.0 mg/L ranged from 2.6% to 8.0%. Samples with concentrations of 0.55mg/L, and 0.93 mg/L were also tested and the CV ranged from 9.1% to 5.3%.

Limit of Quantitation

The LOB, LOD and LOQ of Diazyme Cystatin C POC Test Kit were determined according to CLSI EP17-A. LOB = 0.045 mg/L; LOD = 0.11 mg/L; LOQ = 0.30 mg/L Cystatin C.

Linearity

Eleven levels of the Cystatin C linearity set were prepared by diluting a whole blood containing about 8 mg/L Cystatin C with saline according to CLSI EP6-A and then were run with Diazyme Cystatin C POC Test Kit in triplicates. After linear regression, the correlation coefficient is R^2 = 0.9977, slope is 0.9643, and y intercept is -0.0456. Diazyme Cystatin C POC Test Kit is linear up to 7.65 mg/L. Analytical measuring range (AMR) is 0.30-7.65mg/L.

Analytical Specificity/Interference

To determine the level of interference from the substances normally present in whole blood, the Diazyme Cystatin C POC Test Kit was used to test two whole blood samples with "low" and "high" Cystatin C concentration spiked with various concentrations of substances following CLSI EP7-A guideline. The results showed that the common interfering substances had less than 10% interference up to the concentrations summarized below:

Interference	Concentration
Triglyceride	1000 mg/dL
Ascorbic Acid	10 mg/dL
Bilirubin	40 mg/dL
Bilirubin Conjugated	40 mg/dL
Rheumatoid Factor	1000 IU/mL
Hemoglobin	10g/dL

Method Comparison

To demonstrate accuracy, the paired human whole blood-plasma samples (a tube of whole blood and a tube of plasma from the same individual) were tested for comparison. The whole blood samples were tested with the Diazyme Cystatin C POC Test Kit on SMART Analyzer and the correspondent plasma samples were tested with Diazyme Cystatin C Assay on Hitachi 917 following CLSI EP9-A2 guideline. A total of 55 whole blood-plasma pairs with Cystatin C concentrations ranging from 0.48 to 6.10 mg/L were tested. The linear regression gave a correlation of r^2 value of 0.9867, slope of 0.9535, and y intercept of 0.0958.

120 whole blood samples were also tested at three POL sites by intended users. Each site ran 40 whole blood samples using SMART analyzers. The corresponding one hundred and twenty (120) plasma specimens were tested on Hitachi 917 with predicate device. The 120 whole blood-plasma pairs contained Cystatin C concentrations ranging from 0.51 to 6.81 mg/L.

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	0.9967	0.9049	0.9617	0.955
Intercept	0.1058	0.0731	0.0352	0.0723
R^2	0.9902	0.9902	0.9937	0.9872

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Diazyme Glycated Serum Protein (GSP) POC Test Kit

Configuration

The Diazyme Glycated Serum Protein POC Test Kit is provided in the following kit configurations:

Instrument	REF	Kit size	
SMART Analyzer (546nm)	DZ112B-SMA	40 Test Kit DRS* Cuvette (Reagent R1) DRS Cap (Reagent R2) RFID card	 40 pcs 40 pcs 1 pc

* DRS: Diazyme Reagent System (DRS)

Intended Use

The Diazyme Glycated Serum Protein POC Test Kit is intended for the quantitative determination of glycated serum proteins (GSP; fructosamine) in serum. Fructosamine is representative of blood glucose levels over the course of 2-3 weeks. The measurement of glycated serum proteins is useful for monitoring diabetic patients. For *in vitro* diagnostic use only.

Clinical Significance[1,5]

Fructosamine is formed due to a non-enzymatic Maillard reaction between glucose and amino acid residues of serum proteins. It is reported that 80% of measured glycated serum proteins are glycated albumins. In diabetic patients, elevated blood glucose levels correlate with increased fructosamine formation. Glycated serum proteins (GSP; fructosamine) are a medium term indicator of diabetic control (2-3 weeks).

Assay Principle[2]

The Diazyme Glycated Serum Protein POC Test Kit uses proteinase K to digest GSP into low molecular weight glycated protein fragments (GPF), and uses Diazyme's specific fructosamiase™, a microorganisms originated amadoriase to catalyze the oxidative degradation of Amadori product GPF to yield peptide fragments (PF) or amino acids, glucosone and H_2O_2. The H_2O_2 released is measured by a colorimetric Trinder end-point reaction. The absorbance at 546 nm is proportional to the concentration of glycated serum proteins. The SMART analyzer calculates the GSP concentrations of patient serum specimens by use of a lot specific calibration curve. The lot specific curve is represented in a Calibration card (RFID) provided with each Diazyme GSP POC Test Kit.

$$\text{GSP} \xrightarrow{\text{Proteinase K}} \text{Glycated protein fragments (GPF)}$$

$$\text{GPF} \xrightarrow{\text{Fructosaminase}} \text{PF or amino acids} + H_2O_2$$

$$H_2O_2 + \text{TOOS} + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Color} + H_2O$$

Reagent Composition

REAGENT 1: Enzyme/substrate reagent containing Tris HCl buffer, 4-AA, Enzymes and stabilizers

REAGENT 2: Enzyme/substrate reagent containing Tris HCl buffer, enzymes, TOOS, HRP, Geneticin, and stabilizers

Materials Required but not Provided

A SMART Analyzer (with a single or dual wavelength) with a 546 nm wavelength. The Diazyme GSP Control Set for validating the performance of the Diazyme GSP reagents are provided separately (REF DZ112B-CON).

Reagent Stability and Storage

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

Specimen Collection and Handling

Serum samples can be used for the Diazyme GSP POC Test Kit. Use fresh patient serum samples. Serum should be separated from cells immediately after collection. Samples can be stored at 2-8°C for 2 weeks or at -20°C for up to 4 weeks. Per CLSI guideline, it is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific specimen stability criteria for its laboratory.[3]

Assay Procedures

The step by step assay procedure is illustrated below:

1. Power the SMART device and open the Diazyme GSP POC Test Kit box (REF DZ112B-SMA) (picture 1).
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 to allow warm up before use.**
4. Add 40 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 on the far 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).

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7. **Caution: 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.** Insert the capped DRS cuvette into the cuvette holder on the door of the SMART analyzer (picture 7).
8. To start the assay, close the door by pressing the Confirm button on the screen (picture 8).. The result is displayed on the screen in approximately 10 min (picture 9).

1.

2.

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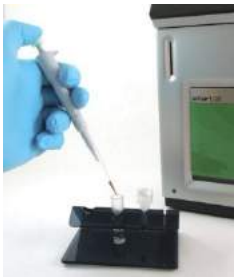

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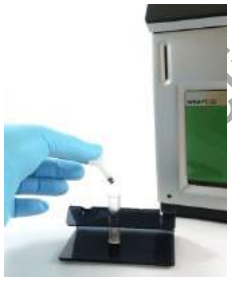

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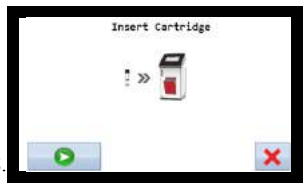

9.

Precautions

1. For *in vitro* diagnostic use only
2. Caution: Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the laws of the State in which he practices, to use or order the use of the device.
3. Only use lot specific RFID card with corresponding reagent lot
4. Store the REAGENT at 2-8°C. Do not freeze the REAGENT.
5. Do not use the REAGENT after the expiration date labeled on the outer box.
6. DO NOT INGEST. Avoid contact with skin and eyes.
7. 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).
8. 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.
9. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.

Quality Control

Diazyme GSP Control Set (REF DZ112B-CON) set can be purchased separately.

The GSP CONTROL can be used to validate the performance of the Diazyme GSP POC Test Kit. Diazyme bi-level GSP controls (REF DZ112B-CON) are recommended. The range of acceptable control limits should be established by individual laboratories.

Quality control weekly and when reagent lot is changed. CONTROL material is to be treated exactly the same as samples when following assay procedure.

Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls and handling of bio-hazardous material.

Results

Results are printed out in µmol/L. The analytical measuring range for this assay is 61 to 1348 µmol/L. Note: Samples with values greater than 1348 µmol/L should be diluted 1:1 with saline and rerun on the SMART analyzer. The result is then multiplied by the dilution factor of 2

Reference Range

Study was performed to determine expected values for non-diabetic adults and the reference range was found to be 191-289 µmol/L. It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population it serves.

Limitations

1. A sample with a GSP level exceeding the linearity limit of 1348 µmol/L should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.
2. Samples that show hemolysis should not be used for testing.

Precision

The precision of the Diazyme GSP POC Test Kit was evaluated according to CLSI EP5-A guideline. In the study, five serum samples at concentrations of 87, 226, 479, 743, and 1244 µmol/L fructosamine, respectively, were tested 2 runs per day in duplicates over 20 working days.

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Within run precision

	Serum Level 1:	Serum Level 2:	Serum Level 3:	Serum Level 4:	Serum Level 5:
n	80	80	80	80	80
Mean	87	226	479	743	1244
SD	5.26	9.75	16.09	12.12	18.60
CV%	6.1%	4.3 %	3.4 %	1.6%	1.5%

With-laboratory Precision

	Serum Level 1:	Serum Level 2:	Serum Level 3:	Serum Level 4:	Serum Level 5:
n	80	80	80	80	80
Mean	87	226	479	743	1244
SD	4.8	10.14	16.48	18.81	22.71
CV%	5.6%	4.5 %	3.4 %	2.5%	1.8%

The 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 SMART Analyzers. At each site, 5 serum samples were tested with three operators. Each sample was run 4 times per day for 5 days. A total of 15 serum samples containing GSP levels ranging across the analytical measuring range were used for the precision study. The Intra Assay CV for samples ranged from 1.80% to 6.27% and total precision yielded a CV range of 1.65% to 7.81%.

Limit of Quantitation

The LOD and LOQ of Diazyme GSP POC Test Kit were determined according to CLSI EP17-A. The LOD was determined to be 30 µmol/L and LOQ was 61µmol/L.

Linearity

Nine levels of linearity set were prepared by diluting a sample containing 1348 µmol/L Fructosamine with low sample with 2 µmol/L Fructosamine according to CLSI EP6-A and analyzed on SMART Analyzer. Results indicated linearity from 61 to 1348 µmol/L. Allowable systematic error (SEA) was 4.9%.

The analytical measuring range (AMR) for this assay is 61 to 1348 µmol/L.

Analytical Specificity/Interference

The following interfering substances produce less than 10% deviation when tested at the indicated concentrations.

Ascorbic Acid	20 mg/dl
Bilirubin	7.5 mg/dl
Bilirubin Conjugated	5 mg/dl
Glucose	2400 mg/dl
Hemoglobin	100 mg/dl
Uric Acid	35mg/dl
Triglyceride	1000 mg/dl
Total Protein	12 mg/dL

Method Comparison

Correlation studies were done by testing 54 human serum samples on the SMART Analyzer and running the same samples in parallel on the Hitachi 917 using commercially available assay. The Passing Bablok regression results are summarized in the following table:

n	54
Slope	0.9737 (95%CI:0.95599-0.9944)
Intercept (µmol/L)	0.6859 (95% CI: -4.7287-7.8918)
Correlation coefficient r	0.9975 (95%CI:0.9956-0.9985)
Range of values (µmol/L)	70.0 to 1269

The method comparison study was performed externally at the three POL sites. One hundred and sixty (160) human serum specimens are tested on with the Diazyme GSP POC Test Kit reagents on SMART analyzers and with a predicate device on Hitachi 917. Passing Bablok regression analysis of the results obtained from the three POL sites is summarized as follows:

SMART GSP	Site 1	Site 2	Site 3	All 3
n	50	60	50	160
Slope	1.05	1.06	1.02	1.04
Intercept	-1.4	6.5	-4.9	-1.3
r	0.99	0.99	0.99	0.99
Range of values	82 to 1330	63 to 1390	65 to 1318	63 to 1390

References

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