

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 20 pcs (Reagent R1) DRS Cap 20 pcs (Reagent R2) 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 Significance¹⁻⁷

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.

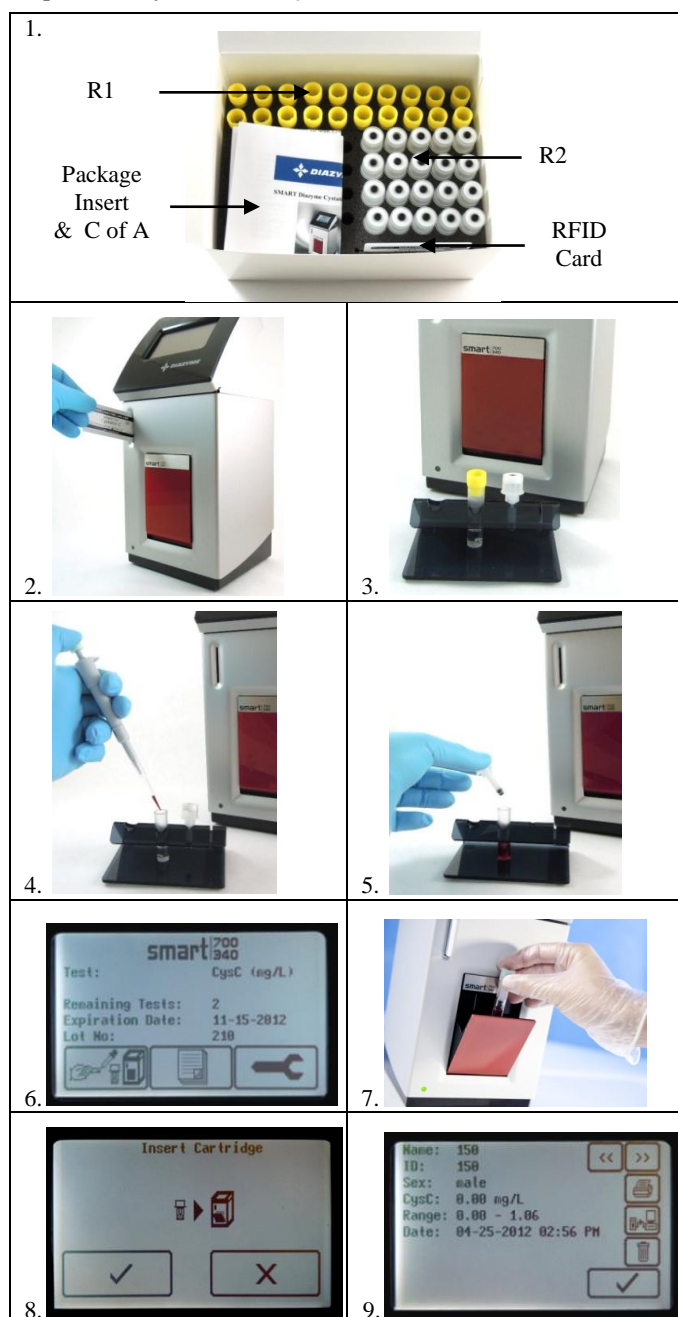
Note: Human specimens and all materials that are in contact with samples should be handled and disposed of according to local and national laws and as if such samples are capable of transmitting infection.

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 equilibrate 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).

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/L⁶. 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.

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 assistants 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

References

1. Filler G, Bökenkamp A, Hofmann W, Le Bricon T, Martínéz-Brú C, Grubb A. Cystatin C as a marker of GFR - history, indications, and future research. Clin Biochem 2005; 38: 1-8.
2. Dharmidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 2002; 40: 221-226.
3. Grubb A, Björk J, Bondesson P, Lindström V, Sterner G, Nyman U. Cystatin C estimates glomerular filtration rate better than creatinine clearance using the Cockcroft-Gault formula. Scand J Clin Lab Invest 2005; 65: 1-10.
4. Thomassen SA, Johannesen IL, Erlandsen EJ, Abrahamsen J, Randers E. Serum cystatin C as a marker of the renal function in patients with spinal cord injury. Spinal Cord 2002; 40: 524-528.
5. Christensson AG, Grubb AO, Nilsson JA, Norrgren K, Sterner G, Sundkvist G. Serum Cystatin C advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy. J Intern Med 2004; 256: 510-518.
6. Alan H.B. Wu. Tietz Clinical Guide to Laboratory Tests. Fourth Ed. Saunders Elsevier, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. 2006; 328-329.
7. Anders Grubb, Ulf Nyman, Jonas Bjork, Veronica Lindstrom, Bengt Rippe, Gunnar Sterner, and Anders Christensson Simple Cystatin C-Based Prediction Equations for Glomerular Filtration Rate Compared with the Modification of Diet in Renal Disease Prediction Equation for Adults and the Schwartz and the Counahan-Barratt Prediction Equations for Children. Clin. Chem. 51:8 1420-1431 (2005).

