AIA-360 Assay Specifications
ST Cystatin C Test Code 102

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 mg/L
3	Cal 2	0.01 mg/L (example)
4	Cal 3	0.04 mg/L (example)
5	Cal 4	0.08 mg/L (example)
6	Cal 5	0.16 mg/L (example)
7	Cal 6	0.35 mg/L (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	mg/L
11	Smpl. Vol	10
12	Dil. Vol	140
13	Assay L	0.0040
14	Assay H	0.3200
15	Ref. L	
16	Ref. H	
17	Decimal	2

Visible only in Test Mode

18	Test Name	#CysC
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	3
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	1.000000
25	Factor1 B	0.000000
26	Factor2 A	25.000000
27	Factor2 B	0.000000
28	V. Conc	0.000000
29	G Origin	0.000000

AIA-900 Assay Specifications
ST Cystatin C Test Code 102

No.	Item	Data
1	Code	102
2	ACT	1
3	Analyte	#CysC
4	Lot	***Enter current cal lot no.
5	CAL 1	0 mg/L
6	CAL 2	0.01 mg/L (example)
7	CAL 3	0.04 mg/L (example)
8	CAL 4	0.08 mg/L (example)
9	CAL 5	0.16 mg/L (example)
10	CAL 6	0.35 mg/L (example)
11	Cal lot L	
12	Cal lot R	
13	Unit	mg/L
14	Decimal	2
15	Assay Low	0.0040
16	Assay High	0.3200
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.1
20	Reschedule High	8.0
21	Factor A	1
22	Factor B	0
23	Sample Volume	10
24	Diluent Volume	140
25	2 Step reagent dispensing volume	0
26	Calibration code	102
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	3
30	CAL. CV	90
31	DIL. SP1	25
32	DIL. SP2	1
33	DIL. CAL. (Calculation of dil ratio of conc.)	1
34	DIL. CNTL.	25
35	DIL. DO	10
36	DIL. AH.	5
37	DIL. CALC.	1
38	DIL. CODE	102
39	DIL. NAME	CysC
40	DIL. PRTY	3
41	PRE. SPVOL (Vol. of pretreated sample)	0
42	PRE. 1VOL (Vol. of pretreatment sol-1)	0
43	PRE. 2 VOL (Vol. of pretreatment sol-2)	0
44	PRE. CODE (pretreated sol. code)	0
45	PRE. NAME (pretreated sol. name)	0
46	Protocol	1
47	SYS. F_A	1
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-2000 Assay Specifications
ST Cystatin C Test Code 102

No.	Item	Data
1	Unit	mg/L
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.1000
6	Reschedule high	8.00000
7	Assay range low	0.0040
8	Assay range high	0.3200
9	Specimen diluent code	102
10	Specimen diluent name	CysC
11	Dilution factor for Sp. 1	25
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	25
14	Default multiplier for DO	10
15	Default multiplier for >H	5
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	003
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal lot no.
21	Calibrator Concentration	
22	Cal - 01	0 mg/L
23	Cal - 02	0.01 mg/L (example)
24	Cal - 03	0.04 mg/L (example)
25	Cal - 04	0.08 mg/L (example)
26	Cal - 05	0.16 mg/L (example)
27	Cal - 06	0.35 mg/L (example)
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	90
36	Incubation time (10/40)	10
37	Assay protocol	1
38	Specimen volume	10
39	Diluent volume	140
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	0
43	Pretreatment specimen volume	0
44	Pretreatment Reagent code	0
45	Pretreatment Reagent name	0
46	Pretreatment Reagent 1 volume	0
47	Pretreatment Reagent 2 volume	0
48	System Factor	1.00
49	Calibration Code Check	102
50	Virtual Concentration	0.00000
51	Graph Origin	0.00000
52	Calculation with Dilution Factor	Yes

Cystatin C
ST AIA-PACK Cystatin C

Name and Intended Use

ST AIA-PACK Cystatin C is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Cystatin C in human serum, heparinized plasma or EDTA plasma on Tosoh AIA System Analyzer. Cystatin C measurement is used as an aid in the diagnosis and treatment of renal disease.

Summary and Explanation of Test

Human Cystatin C is a basic protein with low molecular mass (13 kDa) in the Cystatin superfamily.

Cystatin C is produced at a constant rate in all nucleated cells.

The function of Cystatin C seems to be to protect connective cell or tissue from destruction by intracellular enzymes. The gene is of the house-keeping type, which is compatible with a stable production rate of Cystatin C by most cell types, even under inflammatory conditions. Cystatin C is not an acute phase reactant [(1).]

Due to its small size, Cystatin C is freely filtered by the glomerulus. It is not secreted but is fully reabsorbed and broken down by the renal tubules.[(2-3)] Therefore, Cystatin C concentrations correlate with the glomerular filtration rate (GFR) better than creatinine. [(4-5)] . Cystatin C increases with the decrease of GFR. [(6)]

The most widely used measures of GFR in clinical practice are based on the 24-hour creatinine clearance or serum creatinine concentration. β2-microglobulin and serum creatinine are also measured as marker of renal function for GFR [(7)].

The measurement of creatinine clearance is inconvenient and burdensome on the patient. It requires a timed urine collection, which may be incomplete for various reasons. Serum creatinine is influenced by multiple non-renal factors, including diet, gender, muscle mass and secretion of creatinine by the tubules. This combination of events can result in an overestimation of GFR.

Because Cystatin C is not influenced by pre-renal factors as above, Cystatin C has been proposed as a new sensitive endogenous marker of renal function for GFR [(8-10)].

Principle of the Assay

The ST AIA-PACK Cystatin C is a two-site immunoenzymometric assay which is performed entirely in the ST AIA-PACK Cystatin C test cups. Cystatin C present in the test sample is bound with monoclonal antibody immobilized on magnetic beads and enzyme-labeled monoclonal antibody. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the Cystatin C concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK Cystatin C, Cat No. 0025217)

5 trays x 20 test cups (ST AIA-PACK Cystatin C Test Cup)

Plastic test cups containing twelve magnetic beads coated with anti-Cystatin C mouse monoclonal antibody and 100 µL of lyophilized anti-Cystatin C mouse monoclonal antibody conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Materials Required But Not Provided

The following materials are not provided but are required to perform Cystatin C analysis using the ST AIA-PACK Cystatin C (Cat. No. 0025217) on the Tosoh AIA System Analyzer. They are available separately from Tosoh.

Materials		**Cat. No.**
AIA-SYSTEMS:		
AIA-360		0019945
AIA-900		0022930
AIA-900 9tray Sorter		0022931
AIA-900 19tray Sorter		0022932
AIA-2000 ST		0022100
AIA-2000 LA		0022101
AIA-PACK		
AIA-PACK SUBSTRATE SET II		0020968
AIA-PACK SUBSTRATE REAGENT II / AIA-PACK SUBSTRATE RECONSTITUENT II		
ST AIA-PACK Cystatin C CALIBRATOR SET		0025317
ST AIA-PACK Cystatin C CALIBRATOR(1)	0 mg/L	
ST AIA-PACK Cystatin C CALIBRATOR(2)	0.01 mg/L (approx.)	
ST AIA-PACK Cystatin C CALIBRATOR(3)	0.04 mg/L (approx.)	
ST AIA-PACK Cystatin C CALIBRATOR(4)	0.08 mg/L (approx.)	
ST AIA-PACK Cystatin C CALIBRATOR(5)	0.16 mg/L (approx.)	
ST AIA-PACK Cystatin C CALIBRATOR(6)	0.35 mg/L (approx.)	
ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION		0025517
AIA-PACK WASH CONCENTRATE		0020955
AIA-PACK DILUENT CONCENTRATE		0020956
SAMPLE CUPS		0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUPS		0020970

AIA-PACK SAMPLE TREATMENT CUPS	0020971

ADDITIONAL REQUIREMENTS: (Except AIA-360)

PIPETTE TIPS (1000/PKG)	019215
TIP RACK (EMPTY)	019216
PRELOADED PIPETTE TIPS (96 TIPS X 50 RACKS)	996010
PRELOADED PIPETTE TIPS (96 TIPS X 5 RACKS)	996005

Only materials obtained from Tosoh should be used. Materials obtained elsewhere should not be substituted since assay performance is based strictly on Tosoh materials.

Warnings and Precautions

- The ST AIA-PACK Cystatin C is intended for in vitro diagnostic use only.
- ℞ only. US Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.
- Inspect the packaging and the exterior of the aluminum pouch or the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- Test cups from different lots or different assays should not be mixed within a tray.
- The ST AIA-PACK Cystatin C, ST AIA-PACK Cystatin C CALIBRATOR SET and ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- The material derived from human origin used in the preparations of the calibrators has been tested by FDA-cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human origin will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
- Do not use beyond the expiration date.
- The ST AIA-PACK Cystatin C has been designed so that the high dose “hook effect” is not a problem for the vast majority of samples. No “hook effect” phenomenon was observed at Cystatin C concentrations corresponding to 121.0 mg/L in undiluted specimens.
- Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
(2 - 8 °C):	
ST AIA-PACK Cystatin C	0025217
ST AIA-PACK Cystatin C CALIBRATOR SET	0025317
ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION	0025517
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
(18 - 25 °C):	
AIA-PACK DETECTOR STANDARDIZATION TEST CUPS	0020970
AIA-PACK SAMPLE TREATMENT CUPS	0020971

ST AIA-PACK Cystatin C test cups may be stored for up to 1 day at 18 - 25 °C. ST AIA-PACK Cystatin C CALIBRATOR SET must be kept tightly sealed and refrigerated at 2 - 8 ° C. After opening, the calibrators are stable for 1 day at 2 - 8 °C. After opening, ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION is stable for 7 days when used for automatic dilution provided it is not on the instrument (18 - 25 °C) for more than 7 hours per day and the bottles are tightly sealed and refrigerated at 2 - 8 °C immediately after use. The sample diluting solution can be used for up to 90 days provided that 1) it is used for manual dilutions ONLY, and 2) the bottles are kept tightly sealed and refrigerated immediately after use. Reconstituted substrate solution is stable for 3 days at 18 - 25 °C or 30 days at 2 - 8 °C. Working diluent and wash solutions are stable for 30 days at 18 - 25 °C. Reagents should not be used if they appear cloudy or discolored.

Specimen Collection and Handling

Serum, sodium heparin plasma or EDTA plasma is required for the assay. Citrated plasma SHOULD NOT BE USED.

When using serum, a venous blood sample is collected aseptically without additives. Store at 18 - 25 °C until a clot has formed (usually 15 - 45 minutes), then centrifuge to obtain the serum specimen for assay.

When using sodium heparin plasma or EDTA plasma a venous blood sample is collected aseptically with the designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.

Specimen types should not be used interchangeably during the serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.

Samples may be stored at 2 - 8 °C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20°C or below for up to 60 days.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to the assay, slowly bring frozen samples to 18 - 25 °C and mix gently.

All patient specimens require dilution prior to analysis. Samples are diluted by 25 fold with ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION. The AIA-900 and AIA-2000 will automatically perform dilutions and calculate the results. For further information regarding instrument operation, consult the specific Tosoh AIA System Operator's Manual. ST AIA-PACK Cystatin C CALIBRATOR SET does not require dilution prior to assay.

The diluted sample required for analysis is 10 µL.

8 µL of the undiluted sample is required to make the 1:25 dilution.

Procedure

1) Common Reagent Preparation

1a) Substrate Solution

Bring all reagents to 18 - 25 °C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the AIA-PACK SUBSTRATE REAGENT II (Lyophilized), mix thoroughly to dissolve the solid material.

1b) Wash Solution

Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 2.5 L.

1c) Diluent

Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 5.0 L.

2) Calibration

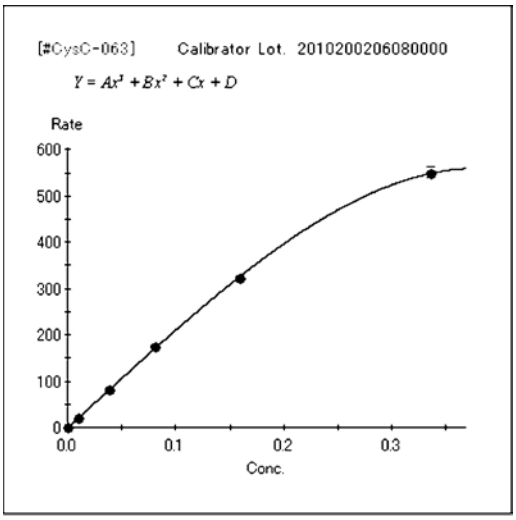
2a) Calibration Curve

The calibrators for use with the ST AIA-PACK Cystatin C are prepared gravimetrically and are compared to internal reference standards.

The calibration curve for the ST AIA-PACK Cystatin C is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and Tosoh AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change). For further information regarding instrument operation, consult the Tosoh AIA System Operator's Manual.

A sample calibration curve below shows the algorithm used to calculate the results. This is an example only. Actual results will vary depending on the instrument type and lot number used. The concentration range of the calibration curve is displayed in 1/25 of the assay range in patient specimens. The concentrations of patient specimens are calculated by multiplying the concentrations obtained on the calibration curve with the dilution factor. The AIA-900 and AIA-2000 will automatically calculate the concentrations of patient specimens using the dilution factor and report the results.



2b) Calibration Procedure

Refer to the appropriate Tosoh AIA System Operator's Manual for the procedural instructions.

i) Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.

ii) Calibrators for ST AIA-PACK Cystatin C are provided ready for use. Tosoh recommends that all calibrators be run in triplicate.

2b) Calibration Acceptability Criteria

i) The mean rate for the ST AIA-PACK Cystatin C CALIBRATOR (1) should be < 2.0 mol/(L.s).

ii) Since there is a direct relationship between concentration and rate, the rate should increase as the concentration increases.

iii) The replicate values should be within a 10% range.

2c) Calibration Review and Acceptance

i) Review the calibration curve carefully, using the criteria listed above.

ii) Edit the calibration if necessary, then accept the calibration.

For further information regarding calibration, consult the specific Tosoh AIA System Operator's Manual.

Quality Control

2d) Commercially Available Controls

Commercially available controls should be run at least once per day. It is recommended that at least two levels of controls, normal Cystatin C and high Cystatin C controls, be used. Laboratory policy for this particular assay designates the following:

Control Material: _____
Frequency: _____

Lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet laboratory criteria will be found in a separate quality control document maintained by the laboratory.

2e) Quality Control Procedure

i) Assay quality control specimens as instructed in the specific Operator's Manual for the Tosoh AIA System Analyzer. In addition, refer to the Tosoh AIA System Operator's Manual for detailed instructions on defining and editing the files.

ii) Quality control should be run in accordance to local, state or federal regulations.

3) Specimen Processing

3a) Preparation

All patient specimens require dilution prior to analysis and are diluted by 25 fold with ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION. Either diluted or undiluted patient specimens are placed in appropriate positions on Tosoh AIA System Analyzer. For further information regarding sample preparation and instrument operation, refer to the Specimen Collection and Handling section and consult the specific Tosoh AIA System Operator's Manual, respectively. Barcoded primary tubes as well as sample cups can be run on the AIA-system analyzer.

3b) Assay Procedure

i) Ensure a sufficient quantity of ST AIA-PACK Cystatin C test cups for the number of samples to be run is available.

ii) Load patient samples as instructed in the Operator's Manual and proceed with analysis. Note: The

iii) The AIA-900 and AIA-2000 will require AIA-PACK SAMPLE TREATMENT CUPs if onboard dilutions are utilized.

Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the Tosoh AIA System Analyzer require that the laboratory use water designated by the CAP Class I or by the clinical laboratory reagent water.

Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the CLSI document GP40-A4-AMD, Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline - Fourth Edition.

3. If a specimen Cystatin C concentration is found to be greater than 8.00 mg/L, the specimen should be diluted with the ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION and reassayed according to the Assay Procedure. The recommended dilution for specimens containing greater than 8.00 mg/L is 5 fold dilution. It is desirable to dilute the specimen so that the diluted specimen reads between 0.1 and 8.00 mg/L. The dilution factor should be entered into the software. For further information on the dilution of specimens, refer to the Tosoh AIA System Operator's Manual.
4. The Tosoh AIA System Analyzer can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK Cystatin C test cups can be used during the same run.
5. If the assay specifications for this test are not ready in the system software, the specifications must be entered under test code 102.

Calculation of Results

The Tosoh AIA System Analyzer performs all sample and reagent handling operations automatically. The Tosoh AIA System Analyzer reads the rate of fluorescence produced by the reaction and automatically converts the rate to Cystatin C concentration in mg/L.

For samples requiring dilution the AIA-900 and AIA-2000 will automatically perform dilutions and calculate results if the dilution factors are entered into the software. For detailed information regarding programming dilutions, consult the appropriate Tosoh AIA System Operator's Manual.

The reportable range for the assay is 0.1 to 8.0 mg/L.

Evaluation of Results

Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples should be assayed daily.

The minimum recommendations for the frequency of running control material are:

1. After calibration, two levels of controls are run in order to accept the calibration curve.

2. The two levels of controls are also repeated after calibration when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).

3. After daily maintenance, at least two levels of the control should be run in order to verify the overall performance of the Tosoh AIA System Analyzer.

If one or more control value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting patient results.

Quality control should be run in accordance to local, state or federal regulations.

Limitations of the Procedure

For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g. symptoms, results of other tests, clinical impressions, therapy, etc.).

Patient samples may also contain human anti-mouse antibody (HAMA) due to either natural antibody production or antibodies produced in response to therapy regimens. HAMA may cause either false positive or false negative results in assays using mouse monoclonal antibodies. Other heterophile antibodies are also known to interfere in assays of this type. ST AIA-PACK Cystatin C has been designed to minimize the effects of heterophile antibodies, but interference from high titers cannot be ruled out.

Results from this or any other in vitro diagnostic procedure which do not correlate with the clinical presentation of the patient should be interpreted with extreme caution.

Using ST AIA-PACK Cystatin C, the highest concentration of Cystatin C measurable by 25-fold dilution with ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION is 0.32 mg/L, which corresponds to 8.00 mg/L in specimens, and the lowest measurable concentration is 0.004 mg/L (assay sensitivity), which corresponds to 0.1 mg/L in specimens.

Although the approximate value of the highest calibrator is 0.35 mg/L, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 0.32 mg/L, which corresponds to approximately 8.00 mg/L in undiluted specimens.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

Samples containing fibrin may exhibit either falsely elevated or falsely decreased results. Fibrin must be eliminated in the sample before assay begins.

Certain medications may interfere with assay performance. Specimens from patients taking medicines and/or medical treatment may show erroneous results. All results should be interpreted with respect to the clinical picture of the patient.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage And Stability, and Procedural Notes sections in this insert sheet.

Expected Values

Each laboratory should determine a reference interval corresponding to the characteristics of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered to the patient.

Reference Ranges

The reference range study was developed with reference to the CLSI protocol entitled: How to Define and Determine Reference Intervals in the Clinical Laboratory (C28-A3).

A total of 149 well-characterized unaltered serum specimens were assayed in singleton utilizing the ST AIA-PACK Cystatin C assay on the AIA-1800 analyzer. The specimens were collected from apparently healthy and ambulatory individuals with a population representative of the United States. Specimens from 123 males between the ages of 19 to 73 years and 26 females between the ages of 22 to 73 years were included in this study.

The reference range specimens had a normal distribution and the central 95% range was defined as the reference interval. The reference range for ST AIA-PACK Cystatin C is 0.50 to 1.16 mg/L.

Number of Samples (n)	149
Reference Interval (Central 95th percentile)	0.50 - 1.16 mg/L

Performance Characteristics

The following performance characteristics were determined using Tosoh AIA-1800 Automated Immunoassay Analyzer.

1) Recovery

1a) Recovery:

Three serum, sodium heparin plasma and EDTA plasma specimens were spiked with three different levels of Cystatin C and assayed before and after spiking.

Sample	Initial Value (mg/L) A	Cystatin C Added (mg/L) B	Expected Value (mg/L) A + B	Measured Value (mg/L)	Percent Recovery (%)
Serum 1	0.96	1.04	2.00	2.05	102.3
	0.96	2.08	3.04	3.07	100.9
	0.96	4.16	5.12	4.95	96.7
Serum 2	0.91	1.00	1.91	2.01	104.9
	0.91	2.00	2.91	3.03	104.2
	0.91	4.01	4.92	4.75	96.6
Serum 3	0.92	1.01	1.93	2.01	104.0
	0.92	2.03	2.95	2.98	101.3
	0.92	4.05	4.97	4.96	99.7
Heparinized Plasma 1	0.91	1.02	1.93	2.03	105.2
	0.91	2.04	2.94	3.08	104.6
	0.91	4.08	4.98	4.86	97.5
Heparinized Plasma 2	1.24	1.05	2.29	2.28	99.5
	1.24	2.10	3.34	3.38	101.1
	1.24	4.20	5.44	4.97	91.3
Heparinized Plasma 3	0.86	1.02	1.88	1.94	103.1
	0.86	2.04	2.90	2.90	100.1
	0.86	4.08	4.94	4.82	97.6
EDTA Plasma 1	0.68	1.54	2.21	2.35	106.2
	0.68	3.07	3.75	3.79	101.0
	0.68	6.15	6.82	6.89	101.0
EDTA Plasma 2	0.69	1.57	2.26	2.32	102.8
	0.69	3.14	3.83	3.94	103.0
	0.69	6.27	6.96	6.65	95.5
EDTA Plasma 3	0.70	1.78	2.48	2.46	99.1
	0.70	3.56	4.26	4.10	96.1
	0.70	7.12	7.82	7.47	95.5

1b) Dilution:

A high Cystatin C sample of approximately 40 mg/L was prepared by spiking an unaltered serum, sodium heparin plasma and EDTA plasma specimen with Cystatin C Antigen. This was considered as the reference specimen (i.e. 100%). Intermediate Cystatin C specimens ranging from approximately 8.0 to 40 mg/L were prepared by spiking the reference specimen into an unaltered serum, sodium heparin plasma and EDTA plasma specimen. These high Cystatin C specimens were then assayed on the AIA-1800 analyzer in 4 replicates with a 1:125 dilution (1:5 in addition to the 1:25 dilution) so that the values fall within the assay range of 0.1 to 8.0 mg/L. The 1:125 dilution was performed automatically by the AIA-1800 instrument.

High Serum Sample Reference sample of conc. approx: 37.95 mg/L	Unaltered Serum Approx conc: 8.65 mg/L	Measured Value on AIA-1800 after multiplying with dilution factor of 125 Mean (A)	Expected Value (B)	Percent Recovery
[%]	[%]	(mg/L)	(mg/L)	(A/B)
100	0	37.95	-	-
75	25	29.16	30.62	95.2
50	50	23.11	23.30	99.2
25	75	16.66	15.98	104.2
0	100	8.65	-	-

High heparinized plasma Sample Reference sample of conc. approx: 38.58 mg/L	Unaltered heparinized plasma Approx conc: 8.68 mg/L	Measured Value on AIA-1800 after multiplying with dilution factor of 125 Mean (A)	Expected Value (B)	Percent Recovery
[%]	[%]	(mg/L)	(mg/L)	(A/B)
100	0	38.58	-	-
75	25	29.82	31.10	95.9
50	50	22.78	23.63	96.4
25	75	16.53	16.15	102.4
0	100	8.68	-	-

High EDTA plasma Sample Reference sample of conc. approx: 38.76 mg/L	Unaltered EDTA plasma Approx conc: 8.85 mg/L	Measured Value on AIA-1800 after multiplying with dilution factor of 125 Mean (A)	Expected Value (B)	Percent Recovery
[%]	[%]	(mg/L)	(mg/L)	(A/B)
100	0	38.76	-	-
75	25	30.35	31.28	97.0
50	50	23.16	23.81	97.3
25	75	16.76	16.33	102.6
0	100	8.85	-	-

1c) Linearity:

The linearity for ST AIA-PACK Cystatin C was determined, based on guidance from CLSI Protocol EP06-A. Serum, sodium heparin plasma and EDTA plasma specimens were used to determine the linearity. The assay has been demonstrated to be linear from 0.1 to 8.00 mg/L, within +/- 10% difference in this interval.

Serum:

Dilution No.	Low : High	Expected Value (mg/L)	Measured Value (mg/L)	CV (%)	Recovery (%)
1	Low Undiluted	0.07	0.07	1.77	100.0
2	9 : 1	0.95	0.95	1.08	100.2
3	8 : 2	1.82	1.81	1.07	99.2
4	7 : 3	2.70	2.67	1.02	99.0
5	6 : 4	3.58	3.49	1.94	97.7
6	5 : 5	4.45	4.34	1.47	97.5
7	4 : 6	5.33	5.17	2.37	97.0
8	3 : 7	6.20	5.91	2.50	95.2
9	2 : 8	7.08	6.87	2.05	97.0
10	1 : 9	7.96	7.59	1.96	95.3
11	High Undiluted	8.83	8.83	1.82	100.0

Sodium heparin plasma:

Dilution No.	Low : High	Expected Value (mg/L)	Measured Value (mg/L)	CV (%)	Recovery (%)
1	Low Undiluted	0.08	0.08	0.67	100.0
2	9 : 1	0.94	0.97	1.80	102.5
3	8 : 2	1.81	1.88	0.73	104.1
4	7 : 3	2.67	2.77	0.44	103.7
5	6 : 4	3.54	3.59	1.52	101.6
6	5 : 5	4.40	4.43	2.36	100.6
7	4 : 6	5.27	5.13	2.58	97.5
8	3 : 7	6.13	5.81	0.77	94.9
9	2 : 8	6.99	6.76	1.43	96.6
10	1 : 9	7.86	7.88	1.43	100.3
11	High Undiluted	8.72	8.72	1.53	100.0

EDTA plasma:

Dilution No.	Low : High	Expected Value (mg/L)	Measured Value (mg/L)	CV (%)	Recovery (%)
1	Low Undiluted	0.07	0.07	1.19	100.0
2	9 : 1	0.89	0.91	2.21	101.7
3	8 : 2	1.71	1.77	2.65	103.6
4	7 : 3	2.53	2.64	1.93	104.3
5	6 : 4	3.35	3.45	0.64	102.9
6	5 : 5	4.17	4.18	2.59	100.3
7	4 : 6	4.99	4.94	0.91	99.1
8	3 : 7	5.81	5.81	1.62	100.1
9	2 : 8	6.63	6.45	2.18	97.4
10	1 : 9	7.45	7.23	2.40	97.1
11	High Undiluted	8.26	8.26	2.90	100.0

2) Precision

The precision study was developed with reference to the CLSI protocol entitled: Evaluation of Precision Performance of Quantitative Measurement Methods (EP05-A2).

Precision was assessed by assaying 2 sets (each set contains 3 levels) of unaltered serum, sodium heparin plasma and 1 set (each set contains 3 levels) of EDTA plasma specimens. One set of serum and sodium heparin plasma specimens was tested on one AIA-1800 instrument using one lot of reagents. Estimates of total and within-run precision were obtained from measurements of 2 replicates in a single run, 2 times a day for 20 non-consecutive days. This equaled to a total of 40 runs and 80 determinants.

The second set of serum and sodium heparin plasma specimens was tested on 2 AIA-1800 instruments each using 2 different lots of reagents. The one set of EDTA plasma was tested on 2 AIA-1800 instruments each using 2 different lots of reagents. Estimates of total and within-run precision were obtained from measurements of 2 replicates in a single run, 2 times a day for 20 non-consecutive days on each instrument. This equaled to a total of 80 runs and 160 determinants. The precision was calculated by using data from both the instruments.

The precision study for the ST AIA-PACK Cystatin C assay was evaluated utilizing 3 AIA-1800 analyzers, 3 different lots of reagents, 4 operators. Each instrument was calibrated with a different set of reagents and the precision data was collected within one calibration cycle on each instrument.

2a) Within run precision:

Sample	Number of determinants	Mean (mg/L)	Pooled Standard Deviation (mg/L)	Coefficient of Variation (CV) (%)
Serum 1A	80	0.70	0.023	3.3
Serum 1B	80	2.15	0.043	2.0
Serum 1C	80	4.69	0.175	3.7
Serum 2A	160	1.15	0.017	1.5
Serum 2B	160	1.75	0.037	2.1
Serum 2C	160	5.11	0.108	2.1
Heparinized Plasma 1A	80	0.69	0.018	2.5
Heparinized Plasma 1B	80	2.08	0.057	2.7
Heparinized Plasma 1C	80	4.69	0.154	3.3
Heparinized Plasma 2A	160	1.10	0.019	1.7
Heparinized Plasma 2B	160	1.77	0.037	2.1
Heparinized Plasma 2C	160	4.93	0.076	1.5
EDTA Plasma 1A	160	0.88	0.013	1.5
EDTA Plasma 1B	160	1.65	0.039	2.4
EDTA Plasma 1C	160	4.87	0.099	2.0

2b) Total precision

Sample	Number of determinants	Mean (mg/L)	Pooled Standard Deviation (mg/L)	Coefficient of Variation (CV) (%)
Serum 1A	80	0.70	0.026	3.7
Serum 1B	80	2.15	0.056	2.6
Serum 1C	80	4.69	0.160	3.4
Serum 2A	160	1.15	0.036	3.1
Serum 2B	160	1.75	0.050	2.9
Serum 2C	160	5.11	0.180	3.5
Heparinized Plasma 1A	80	0.69	0.022	3.1
Heparinized Plasma 1B	80	2.08	0.063	3.0
Heparinized Plasma 1C	80	4.69	0.160	3.4
Heparinized Plasma 2A	160	1.10	0.042	3.8
Heparinized Plasma 2B	160	1.77	0.055	3.1
Heparinized Plasma 2C	160	4.93	0.151	3.1
EDTA Plasma 1A	160	0.88	0.045	5.1
EDTA Plasma 1B	160	1.65	0.060	3.6
EDTA Plasma 1C	160	4.87	0.163	3.3

Correlation

The methods comparison study was developed with reference to the CLSI protocol entitled: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2).

a. Alternate Method Comparison

A total of 140 well-characterized serum specimens (129 unaltered and 11 diluted specimens) were assayed in singleton utilizing the ST AIA-PACK Cystatin C assay on the AIA-1800 analyzer and the alternate method. The regression analysis for the correlation between the alternate method (x) and ST AIA-PACK Cystatin C (y) is as follows:

Regression Analysis		
	Deming	**Regular**
Slope:	0.984 (0.972 to 0.996)	0.981 (0.969 to 0.993)
Intercept:	0.072 (0.037 to 0.106)	0.077 (0.043 to 0.111)
95% Confidence Intervals are shown in parentheses		
Corr Coef (R):	1.00	
Bias:	0.0352	
Points (Plotted/Total):	140/140	
Result Ranges:	0.26 to 7.99 mg/L	

b. Matrix Comparison – Serum vs sodium heparin plasma

A total of 117 well-characterized serum and sodium heparin plasma specimens (106 unaltered and 11 diluted samples) were assayed in singleton utilizing the ST AIA-PACK Cystatin C assay on the AIA-1800 analyzer. Sodium Heparin tubes were used to collect the plasma specimens. The regression analysis of the correlation between serum (x) and sodium heparin plasma (y) on ST AIA-PACK Cystatin C is as follow:

Regression Analysis		
	Deming	**Regular**
Slope:	0.981 (0.972 to 0.990)	0.980 (0.971 to 0.989)
Intercept:	0.015 (-0.005 to 0.035)	0.017 (-0.003 to 0.037)
95% Confidence Intervals are shown in parentheses		
Corr Coef (R):	1.00	
Bias:	-0.0156	
Points (Plotted/Total):	111/111	
Result Ranges:	0.1 to 7.92 mg/L	

c. Matrix Comparison – Serum vs EDTA plasma

A total of 143 well-characterized Serum and EDTA plasma specimens (132 unaltered and 11 diluted samples) were assayed in singleton utilizing the ST AIA-PACK Cystatin C assay on the AIA-1800 analyzer. The regression analysis of the correlation between serum (x) and EDTA plasma (y) on ST AIA-PACK Cystatin C is as follows:

Regression Analysis		
	Deming	**Regular**
Slope:	0.987 (0.980 to 0.993)	0.986 (0.980 to 0.993)
Intercept:	0.018 (0.001 to 0.036)	0.020 (0.002 to 0.037)
95% Confidence Intervals are shown in parentheses		
Corr Coef (R):	1.00	
Bias:	-0.007	
Points Plotted/Total):	137/137	
Result Ranges:	0.1 to 7.87 mg/L	

d. Method Comparison – AIA-1800 vs AIA-360

A total of 61 well-characterized unaltered serum specimens were assayed in singleton utilizing the ST AIA-PACK Cystatin C assay on the AIA-1800 and the AIA-360. The regression analysis of the correlation between AIA-1800 (x) and AIA-360 (y) for the ST AIA-PACK Cystatin C is as follows:

Regression Analysis		
	Deming	**Regular**
Slope:	0.987 (0.965 to 1.009)	0.984 (0.962 to 1.005)
Intercept:	-0.066 (-0.129 to -0.003)	-0.058 (-0.121 to 0.005)
95% Confidence Intervals are shown in parentheses		
Corr Coef (R):	1.00	
Bias:	-0.0962753	
Points (Plotted/Total):	61/61	
Result Ranges:	0.50 to 7.43 mg/L	

e. Method Comparison – AIA-1800 vs AIA-600II

A total of 61 well-characterized unaltered serum specimens were assayed in singleton utilizing the ST AIA-PACK Cystatin C assay on the AIA-1800 and the AIA-600II. The regression analysis of the correlation between AIA-1800 (x) and AIA-600II (y) for the ST AIA-PACK Cystatin C is as follows:

Regression Analysis		
	Deming	**Regular**
Slope:	1.025 (0.998 to 1.052)	1.020 (0.993 to 1.047)
Intercept:	-0.105 (-0.183 to -0.026)	-0.092 (-0.170 to 0.014)
95% Confidence Intervals are shown in parentheses		
Corr Coef (R):	1.00	
Bias:	-0.0453231	
Points (Plotted/Total):	61/61	
Result Ranges:	0.50 to 7.51 mg/L	

f. Method Comparison – AIA-1800 vs AIA-2000

A total of 61 well-characterized unaltered serum specimens were assayed in singleton utilizing the ST AIA-PACK Cystatin C assay on the AIA-1800 and the AIA-2000. The regression analysis of the correlation between AIA-1800 (x) and AIA-2000 (y) for the ST AIA-PACK Cystatin C is as follows:

Regression Analysis		
	Deming	**Regular**
Slope:	0.967 (0.965 to 1.009)	0.984 (0.962 to 1.005)
Intercept:	-0.066 (-0.129 to -0.003)	-0.058 (-0.121 to 0.005)
95% Confidence Intervals are shown in parentheses		
Corr Coef (R):	1.00	
Bias:	-0.0962753	
Points (Plotted/Total):	61/61	
Result Ranges:	0.55 to 6.53 mg/L	

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The Limit of Detection (LoD) for AIA-PACK Cystatin C is 0.0012mg/L, determined consistent with the guidelines in the CLSI protocol EP 17-A and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 120 determinations, with 60 blank and 60 low level samples. LoB is 0.0011mg/L. The goal for Total Error (TE) was 0.005 mg/L and the calculated TE was 0.0004 mg/L. Since the TE was less than the goal LoQ = LoD. Therefore LoQ is 0.0012mg/L.

The reportable range for the assay is 0.1 to 8.0 mg/L.

Interference

The criteria for no interference are +/- 10% (90-110%) recovery of Cystatin C of the known specimen mean concentration.

- Hemoglobin (up to 470 mg/dL), free bilirubin (up to 19 mg/dL) and conjugated bilirubin (up to 18 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by triglyceride concentration (up to 1,600 mg/dL), does not interfere with the assay.
- Protein, as indicated by human albumin concentrations (up to 5.0 g/dL), did not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
- Rheumatoid factor (up to 1,300 IU/mL) does not interfere with the assay.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.

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1. Abrahamson M, Olafsson I, Palsdottir A, Ulvsback M, Lundwall A, Jensson O, Grubb A : Structure and expression of the human Cystatin C gene. Biochem J 1 : 287-294 (1990).
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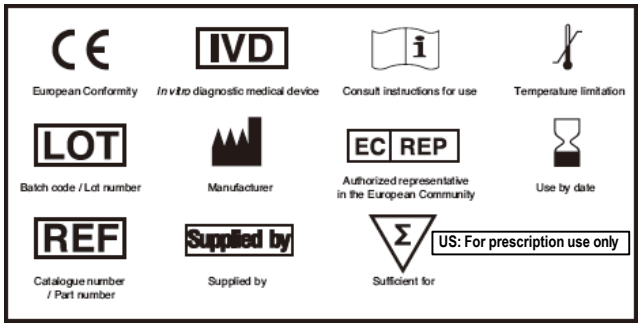

ST AIA-PACK
Cystatin C CALIBRATOR SET

Intended Use

The ST AIA-PACK Cystatin C CALIBRATOR SET is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration of the ST AIA-PACK Cystatin C assay.

Summary and Explanation

The ST AIA-PACK Cystatin C CALIBRATOR SET contains buffered bovine serum albumin with assigned levels of Cystatin C. Cystatin C from human origin is used in the preparation of these calibrators. Calibration should be performed according to the schedule indicated in the Tosoh AIA System Operator's Manual.

Material Provided (Cat. No. 0025317)

2 x 1 mL	ST AIA-PACK Cystatin C CALIBRATOR (1)	0.0	mg/L

Buffered bovine serum albumin containing no detectable concentration of Cystatin C with 0.1% sodium azide as a preservative. (Ready to Use)

2 x 1 mL	ST AIA-PACK Cystatin C CALIBRATOR (2)	0.01	mg/L (approx.)
	ST AIA-PACK Cystatin C CALIBRATOR (3)	0.04	mg/L (approx.)
	ST AIA-PACK Cystatin C CALIBRATOR (4)	0.08	mg/L (approx.)
	ST AIA-PACK Cystatin C CALIBRATOR (5)	0.16	mg/L (approx.)
	ST AIA-PACK Cystatin C CALIBRATOR (6)	0.35	mg/L (approx.)

Buffered bovine serum albumin containing the assigned concentration of Cystatin C (described on each vial) with 0.1% sodium azide as a preservative. (Ready to Use)

Warnings and Precautions

1. The ST AIA-PACK Cystatin C CALIBRATOR SET is for in vitro diagnostic use.
2. This material contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.

3. The material derived from human origin used in the preparations of these calibrators has been tested by FDA-cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human origin will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
4. Do not use beyond the expiration date.

Preparation of Reagents

The ST AIA-PACK Cystatin C CALIBRATOR SET is provided ready for use. Bring the calibrators to 18 - 25 °C for use.

The ST AIA-PACK Cystatin C Calibrator materials do not require dilution prior to assay.

Storage and Stability

When stored unopened and refrigerated at 2 - 8 °C, the ST AIA-PACK Cystatin C CALIBRATOR SET is stable until the expiration date on the label. Calibrator materials should be used within 1 day after opening, provided the vials are kept tightly sealed and refrigerated at 2 - 8 °C.

Procedure

Refer to the Calibration Procedure in the AIA-PACK section of this analyte application. For additional procedural instructions regarding calibration, refer to the Tosoh AIA System Operator's Manual.

1. Verify that both the calibrator lot and concentrations have been correctly entered into the analyzer software.
2. Add the appropriate amounts of each calibrator to sample cups (refer to the instrument worklist for the amount required in each sample cup).
3. Place the sample cups and the test cups on the analyzer as indicated on the worklist.
4. Tosoh recommends that all calibrators be run in triplicate.

Assignment of Values

The ST AIA-PACK Cystatin C CALIBRATOR SET contains assigned concentrations of Cystatin C. The assigned value is determined on a lot-by-lot basis and is designed to provide an assay calibration range of 0.004 to 0.32 mg/L of Cystatin C. The calibrators in this set are prepared gravimetrically and are compared to internal reference standards.

Results

1. The mean rate for the CALIBRATOR (1) should be ＜ 2.0 mol/(L.s).
2. Since there is a direct relationship between concentration and rate, the rate should increase as the concentration increases.
3. The replicate values should be within a 10 % range.

Limitations

The ST AIA-PACK Cystatin C CALIBRATOR SET is designed solely for use with ST AIA-PACK Cystatin C assay procedures. Although the approximate value of the highest calibrator is 0.35 mg/L, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 0.32 mg/L, which corresponds to approximately 8.00 mg/L in undiluted specimens.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.

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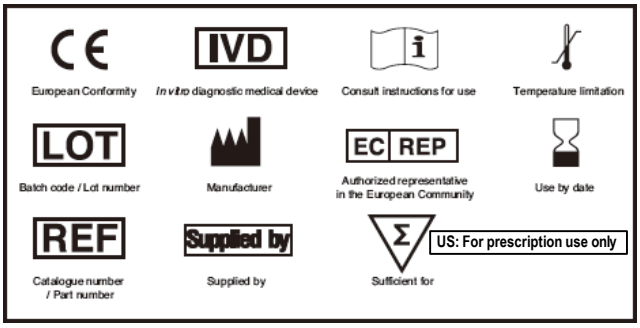

ST AIA-PACK
Cystatin C SAMPLE DILUTING SOLUTION

Intended Use

The ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples.

Summary and Explanation

The ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION contains a bovine protein matrix with no detectable concentration of Cystatin C. This sample diluting solution is to be used only with samples that are being tested for Cystatin C concentrations using the ST AIA-PACK Cystatin C assay.

Materials Provided (Cat. No. 0025517)

4 x 100mL ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION

Buffered bovine serum albumin containing no detectable concentration of Cystatin C with sodium azide as a preservative.

Warnings and Precautions

1. The ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION is intended for in vitro diagnostic use.
2. This material contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
3. Although material derived from human origin is not used for the sample diluting solution, it is recommended that this product be handled with the same precautions as used for patient samples.
4. Do not use beyond the expiration date.

Preparation and Storage

- The ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION is provided ready for use.
- Always store the sample diluting solution upright as refrigerated at 2 - 8 °C when not in use.

Stability

When stored unopened and refrigerated at 2 - 8 °C, ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION is stable until the expiration date on the label. The sample diluting solution should be used within 7 days as far as it is opened for up to 7 hours at 18 - 25 °C in a day and the bottles are kept tightly sealed and refrigerated at 2 - 8 °C immediately after use. They should be used after equilibrating to 18 - 25 °C for about 30 minutes. After opening, the Sample Diluting Solution can be used for up to 90 days provided that 1) it is used for manual dilutions ONLY, and 2) the bottles are kept tightly sealed and refrigerated immediately after use.

Procedure

Refer to the Tosoh AIA System Operator's Manual for additional procedural instructions regarding sample dilution.

1. Serum, sodium heparin plasma or EDTA plasma samples should be diluted by 25 fold prior to analysis.
2. If a specimen Cystatin C concentration is found to be greater than 8.00 mg/L, the specimen should be diluted with the ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION and assayed according to the Procedure in the insert sheet of the ST AIA-PACK Cystatin C.
3. The AIA-900 and AIA-2000 will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
4. The recommended dilution for specimens containing greater than 8.00 mg/L is 5 fold dilution. It is desirable to dilute the specimen so that the diluted specimen reads between 0.1 and 8.00 mg/L.

Results

When an auto-dilution is performed, the AIA instrument will calculate the final result.

Limitations

The ST AIA-PACK Cystatin C SAMPLE DILUTING SOLUTION is designed solely for use with ST AIA-PACK Cystatin C assay procedures.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.