Diabetes

ichroma™ Cystatin C

INTENDED USE

ichroma™ Cystatin C is a fluorescence immunoassay (FIA) for the quantitative determination of Cystatin C in human serum/plasma. It is useful as an aid in management and monitoring of renal disease.

For *in vitro* diagnostic use only.

INTRODUCTION

The level of serum Cystatin C has been proposed as a simple, accurate, and rapid endogenous marker of glomerular filtration rate (GFR) in research and clinical practice. The measurement of serum Cystatin C may detect mild to moderate decrease in GFR that are not evident with the serum creatinine measurement.

In kidney transplant patients, Cystatin C was reported to be more sensitive than serum creatinine for detecting decreases in GFR and delayed graft function, offering an opportunity for timely intervention.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized-antibodies on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for ichroma™ tests to show Cystatin C concentration in the sample.

COMPONENTS

ichroma™ Cystatin C consists of 'cartridges', 'detector tubes' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has anti-human Cystatin C at the test line, and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has a granule containing anti-human Cystatin C-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, bovine serum albumin (BSA), sucrose as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative. All detector tubes are packed in a pouch.
- The detector diluent contains bovine serum albumin (BSA) as a stabilizer, tween 20 as a detergent, sodium azide in phosphate buffered saline (PBS), and it is pre-dispensed in 2 vials. The detector diluents are packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges or detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the test cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and the detector diluent contain sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in ichroma™ Cystatin C when biotin concentration in the sample was below 1,500ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ Cystatin C** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Cystatin C** should be used only in conjunction with instrument for ichroma™ tests
  - Have to use recommended anticoagulant sample.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, NA_2 EDTA, Lithium heparin, Sodium citrate

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2-30 °C	20 months	Disposable
Detector tube	2-30 °C	20 months	Disposable
Detector diluent	2-30 °C	20 months	Unopened
	2-30 °C	12 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative results as it makes antigen unrecognizable by the antibodies.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-43

Components of **ichroma™ Cystatin C**

- Cartridge Box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 2
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Cystatin C**.

Please contact our sales division for more information.

- **Instrument for ichroma™ tests**
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M3** REF FPRR035
- **Printer** REF FPRR007

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Cystatin C** is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (serum, plasma) may be stored for two weeks at 2-8 °C prior to being tested. If testing will be delayed more than two weeks, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ Cystatin C**: Sealed cartridges, detector tubes, a detector diluent, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tubes, the detector diluent as well as an ID chip.
- If the sealed cartridge, detector tube, and detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for ichroma™ tests.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the 'Instrument for ichroma™ tests operation manual' for complete information and operating instructions.**

TEST PROCEDURE

▶ **ichroma™ Reader, ichroma™ II & ichroma M3**

Multi test mode

1) Take 500 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
   (The detection buffer must be used immediately. Do not exceed 30 seconds.)
2) Take 10 µL of sample (serum/plasma) using a pipette and dispense it to the detector tube.
3) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times
   (The sample mixture must be used immediately. Do not exceed 30 seconds.)
4) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
5) Leave the cartridge at room temperature for 10 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
6) To scan the sample-loaded cartridge, insert it into the

cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
7) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M3 is tested automatically after inserting.)
8) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

Single test mode

1) The test procedure is same with the 'Multi test mode 1) – 4)'.
2) Insert the sample-loaded cartridge into the holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
3) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ tests.
(ichroma™ M3 is tested automatically after inserting.)
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 10 minutes.
5) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ **ichroma™ III**

1) The test procedure is same with the 'Single test mode'.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays Cystatin C concentration of the test sample in terms of mg/L.
- Cut-off (reference range)

Concentration of cystatin C in healthy individuals

Age range	Reference range
19-49 years old	0.53-0.92 mg/L
50-70 years old	0.58-1.02 mg/L

- Concentration of Cystatin C vs. GFR

Stage	Cystatin C (mg/L)	GFR (ml/min/1.73m^2)	State
Normal	0.52-0.91	≥ 90	Normal GFR
1	0.91-1.1	≥ 90	Kidney damage with normal
2	1.1-1.7	60-89	Mild Decrease
3	1.7-2.5	30-59	Moderate Decrease
4	2.5-4.0	15-29	Severe Decrease
5	> 4.0	< 15	ESRD (Kidney failure)

- Prognosis of CKD by GFR and albumin categories

		Albuminuria categories		
		A1	A2	A3
Stage	GFR	< 30 mg/L	30-300 mg/L	> 300 mg/L
1	≥90	Low risk	Medium risk	High risk
2	60-89			
3	45-59	Medium risk	High risk	Very high risk.
4	30-44	High risk		
5	15-29			
6	<15			

- Working range : 0.1-7.5 mg/L

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 0.06 mg/L
  - Limit of Detection (LoD) 0.10 mg/L
  - Limit of Quantitation (LoQ) 0.10 mg/L

- **Analytical specificity**
  - Cross-reactivity

Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ Cystatin C** test result did not show any significant cross-reactivity with these biomolecules.

Interference material	Concentration
TSH	100 uIU/mL
CRP	300 mg/L
hCG	50,000 mIU/mL
PCT	100 ng/mL
TnI	20,000 pg/mL

  - Interference

Interference materials such as below the ones in the table were added to the test sample(s) the same as the below concentrations. **ichroma™ Cystatin C** test result did not show any significant interference with these materials.

Interference material	Concentration
Acetaminophen	20 mg/dL
L-ascorbic acid	500 mg/dL
Bilirubin [conjugated]	2 g/dL
D-glucose	1,000 mg/dL
Intralipid	8,000 U/L
Triglyceride	327 M
Urea	10 g/dL
Biotin	1,500 ng/ml

- **Precision**

Single-site study

Repeatability (within-run precision)

Total precision (within-laboratory precision)

Lot to lot precision

3 Lots of **ichroma™ Cystatin C** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Multi-site study

Between person

3 Lot of **ichroma™ Cystatin C** was tested in 3 different testers.

Between site

1 Lot of **ichroma™ Cystatin C** was tested in 3 different sites.

Between reader

1 Lot of **ichroma™ Cystatin C** was tested in 3 different instruments.

Cystatin C [mg/L]	Repeatability		Total precision		Lot to lot precision	
	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
0.5	0.52	6.33	0.52	6.72	0.51	7.11
1	1.19	4.82	1.17	5.24	1.13	6.18
2	2.07	5.19	2.06	5.33	2.16	6.21

Cystatin C [mg/L]	Between-site		Between-person		Between-reader	
	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
0.5	0.51	5.60	0.52	5.23	0.53	6.43
1	1.12	5.58	1.05	5.02	1.04	5.73
2	2.17	5.25	2.10	6.01	2.08	6.33

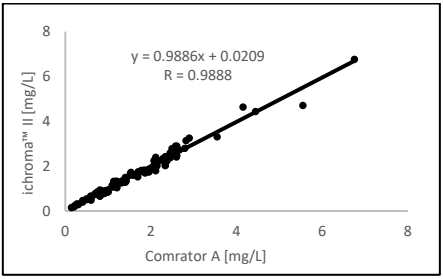
- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of ichroma™ Cystatin C. The tests were repeated 10 times at each concentration of the control standard.

Sample	Expected value [mg/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
Cal. 1	0.1	0.10	0.11	0.11	0.11	105%
Cal. 2	0.5	0.47	0.46	0.47	0.46	93%
Cal. 3	1.0	1.06	1.08	1.09	1.08	108%
Cal. 4	2.0	2.18	2.11	2.11	2.13	107%
Cal. 5	5.0	4.59	4.57	4.63	4.60	92%
Cal. 6	7.5	7.04	8.16	7.14	7.45	99%

- **Comparability**

Cystatin C concentrations of 100 clinical samples were quantified independently with **ichroma™ Cystatin C** (**ichroma™ II**) and **comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



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Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
	Batch code
	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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