ichroma™ Cystatin C

INTENDED USE
ichroma™ Cystatin C along with the ichroma™ Reader is intended for use in a fluorescence immunoassay for quantitative determination of cystatin C in human serum/plasma. The measurement of cystatin C is used as an aid in the diagnosis and treatment 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 immune-detection method, such that the detection antibody in a detection buffer binds to cystatin C in a sample and antigen-antibody complexes are captured to another cystatin C antibody that has been immobilized on a test strip as sample mixture migrates through a nitrocellulose matrix. Thus the more cystatin C antigen in a sample, the more antigen-antibody complexes are accumulated on a test strip. Signal intensity of fluorescence on detection antibody reflects the amount of antigen captured and is processed by ichroma™ Reader to show the cystatin C concentration in a specimen.

• Reference range for the ichroma™ Cystatin C

<table>
<thead>
<tr>
<th>Concentration of cystatin C vs. GFR</th>
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<tbody>
<tr>
<td>Stage</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>Normal</td>
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<tr>
<td>1</td>
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<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration of cystatin C in healthy individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>18 - 50 years old</td>
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<tr>
<td>51 - 70 years old</td>
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</table>

<table>
<thead>
<tr>
<th>Prognosis of CKD by GFR and albuminuria categories</th>
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<tbody>
<tr>
<td>Stage</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>A1</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>5</td>
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<td>6</td>
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COMPONENTS AND REAGENTS
ichroma™ Cystatin C consists of a ‘test cartridge’, an ‘ID chip’, and a ‘detection buffer tube’
- The test cartridge contains a test strip on which both murine antibodies against human cystatin C and chicken IgY have been immobilized at the test line and the control line, respectively.
- Each test cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed test cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains fluorochrome-labeled anti-cystatin C antibodies, fluorescence-labeled anti-chicken IgY, bovine serum albumin (BSA) as a stabilizer and sodium azide in PBS as a preservative.
- The detection buffer is dispensed in each detection buffer tube. 25 detection buffer tubes are packed in a separate box which is further packed in a styrofoam box provided with ice packs for the purpose of shipment.

WARNINGS AND PRECAUTIONS
- For in vitro diagnostics use only.
- Carefully follow instructions and procedures described in this insert as well as the ichroma™ Reader operation manual.
- Lot numbers of all the test components (test cartridge, ID chip and detection buffer) must match with each other.
- Do not interchange the test components from different lots or use the test components beyond the expiration date.
- Test performed by using any test component with mismatching lot number or that beyond the expiration date may yield misleading test result(s).
- The test cartridge should remain sealed in its original pouch until use. Do not use the test cartridge that is damaged or already opened.
- The detection buffer should attain room temperature prior to performing the test.
- ichroma™ Cystatin C as well as the ichroma™ Reader should be used away from vibration and/or magnetic field. During normal usage, the ichroma™ Reader may produce minor vibrations which should be regarded as normal.
- A detection buffer tube should be used for processing one sample only. Similarly a test cartridge should be used for testing one processed sample only. Both the detection buffer tube and the test cartridge should be discarded after single use.
- Used detection buffer tubes, pipette tips, and test cartridges should be handled carefully and disposed of by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.

STORAGE AND STABILITY
- The test cartridge is stable for 20 months while sealed in an aluminum foil pouch, if stored at 4-30°C.
- The detection buffer dispensed in a tube is stable for 20 months, if stored at 2-8°C.
- After the test cartridge pouch is opened, the test should be performed immediately.

LIMITATIONS OF THE TEST SYSTEM
ichroma™ Cystatin C provides accurate and reliable test results subject to the following constraints:
- ichroma™ Cystatin C should be used only in conjunction with the ichroma™ Reader.
- The test should always be performed on freshly collected serum or plasma sample(s).
- Plasma and serum with or without anticoagulants (EDTA, heparin and sodium citrate) can be used.
- The test sample must be at room temperature prior to testing. If the test samples are to be shipped for the purpose of this test, appropriate precautions must be exercised.
- Effectiveness of the test is highly dependent on storage conditions of test components and test samples.
- The test may yield false positive results due to cross-reactivity of some components of serum with the capture/detection antibodies.
- The test may yield false negative results due to cystatin C epitope being masked by some unknown components. False negative results may also be obtained due to instability or degradation of the cystatin C antigen with time and/or temperature.
- Other factors interfering with the test and causing erroneous results include technical/procedural errors, degradation of the test components/reagents as well as presence of interfering substances in the test samples.
- 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.

SAMPLE COLLECTION AND PROCESSING
The test can be performed on either serum or plasma.
- It is strongly recommended to test the sample within 24 hours after collection.
- The serum and plasma should be prepared by centrifugation within 3 hours after the collection of whole blood.
- If the test cannot be performed within 24 hours after preparation of test samples, they should be immediately frozen below -20 degrees, and it’s allowed to keep them in a freezer for 3 months only.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the decrease in test values.

MATERIALS SUPPLIED
REF CFPC-43
Components of ichroma™ Cystatin C
- Test Cartridge Box
  - Sealed test cartridges 25
  - ID chip 1
  - Package insert 1
- Box containing Detection Buffer Tube
  - Detection buffer tubes 25
MATERIALS REQUIRED BUT SUPPLIED UPON DEMAND

Following items can be purchased separately from ichroma™ Cystatin C. Please contact our sales division for more information.
- ichroma™ Reader REF FR203
- Thermal Printer

TEST SETUP

1. Check the contents of the ichroma™ Cystatin C: Sealed Test Cartridge, ID Chip, and detection buffer tube.
2. Ensure that the lot number of the test cartridge matches with that of the ID chip as well as the detection buffer tube.
3. Keep the sealed test cartridge and the detection buffer tube at room temperature for at least 30 minutes just prior to the test, if stored in refrigerator. Place the test cartridge on a clean, dust-free and flat surface.
4. Turn on the power supply of the ichroma™ Reader.
5. Insert the ID Chip into the ID chip port of the ichroma™ Reader.
6. Press the ‘Select’ button on the ichroma™ Reader.

Operation condition for the ichroma™ Cystatin C
- Temperature: 20-30°C
- Humidity: <70%

TEST PROCEDURE

1. Transfer 10 µL of the serum or plasma sample using a transfer pipette to a tube containing the detection buffer.
2. Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 10 to 15 times.
3. Take 75 µL of a sample mixture and dispense it into the sample well on the test cartridge.
4. Leave the sample-loaded test cartridge at room temperature for 10 minutes.
5. For scanning, insert it into the test cartridge holder of the ichroma™ Reader. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder. An arrow has been marked on the test cartridge especially for this purpose.
6. Press ‘Select’ button on the ichroma™ Reader to start the scanning process.
7. The ichroma™ Reader will start scanning the sample-loaded test cartridge immediately.
8. Read the test result on the display screen of the ichroma™ Reader.

INTERPRETATION OF TEST RESULT

- ichroma™ Reader calculates the test result automatically and displays cystatin C concentration of the test sample as mg/L.
- Working range of ichroma™ Cystatin C is 0.1-7.5 mg/L
- ichroma™ Cystatin C test should be considered as a screening tool only. Please consult a physician to discuss the test result. The physician may decide further course of action.

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Before testing a clinical sample using a new test lot, control reagents should be tested to confirm the test procedure, and to verify whether the test produces the expected results.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control reagents are not provided with the ichroma™ Cystatin C. For more information regarding obtaining the control reagents, contact Boditech Med Inc.’s Technical Services for assistance.
- ichroma™ Cystatin C test has a built-in internal control that satisfies the routine quality control requirements. This internal control test is performed automatically each time a clinical sample is tested. An invalid result from the internal control leads to display an error message on the ichroma™ Reader indicating that the test should be repeated.

PERFORMANCE CHARACTERISTICS

1. Interference: Study of interference from EDTA, urea, sodium citrate, glucose, heparin, with ichroma™ Cystatin C showed the following results.

<table>
<thead>
<tr>
<th>Interference</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>EDTA</td>
<td>100 mg/ml</td>
</tr>
<tr>
<td>Urea</td>
<td>2 mg/ml</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>22 mg/ml</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>10 mg/ml</td>
</tr>
<tr>
<td>Heparin</td>
<td>10 KU/ml</td>
</tr>
</tbody>
</table>

There was no significant interference from these materials with the ichroma™ Cystatin C test measurements.

2. Prozone/Hook Effect: No prozone/hook effect was observed with ichroma™ Cystatin C at cystatin C
3. **Precision:** For studying intra-assay imprecision, 10 replicates of each of the three concentrations of control reagent were tested. For studying inter-assay imprecision, 10 replicates of each of the three concentrations of control reagent were tested by three different persons.

<table>
<thead>
<tr>
<th>Cystatin C [mg/L]</th>
<th>Intra-assay Mean</th>
<th>CV (%)</th>
<th>Inter-assay Mean</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.48</td>
<td>3.11</td>
<td>0.48</td>
<td>2.66</td>
</tr>
<tr>
<td>1</td>
<td>0.99</td>
<td>1.38</td>
<td>0.98</td>
<td>3.18</td>
</tr>
<tr>
<td>2.5</td>
<td>2.35</td>
<td>1.84</td>
<td>2.32</td>
<td>3.04</td>
</tr>
</tbody>
</table>

4. **Comparability:** Cystatin C concentrations of 231 plasma samples were quantified independently with the ichroma™ Cystatin C and Roche_Modular per prescribed test procedures. Test results were compared and their compatibility was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=0.93334X + 0.14119 and R=0.980, respectively.

![Graph showing linear regression](image)

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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Read instructions for use</td>
<td></td>
</tr>
<tr>
<td>Use by</td>
<td></td>
</tr>
<tr>
<td>Batch code</td>
<td></td>
</tr>
<tr>
<td>Catalog number</td>
<td></td>
</tr>
<tr>
<td>Caution</td>
<td></td>
</tr>
<tr>
<td>Manufacturer</td>
<td></td>
</tr>
<tr>
<td>Authorized representative of the European Community</td>
<td></td>
</tr>
<tr>
<td>In vitro diagnostic medical device</td>
<td></td>
</tr>
<tr>
<td>Temperature limit</td>
<td></td>
</tr>
<tr>
<td>Do not reuse</td>
<td></td>
</tr>
<tr>
<td>This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices</td>
<td></td>
</tr>
</tbody>
</table>

For technical assistance; please contact:

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**REFERENCES**