**INTENDED USE**

**ichroma™ IL-6** is a fluorescence Immunoassay (FIA) for the quantitative detection of IL-6 in human whole blood/serum/plasma. It is helpful as an aid in management and monitoring of inflammatory disease.

For *in vitro* diagnostic use only.

**INTRODUCTION**

IL-6 (Interleukin-6) is produced by a variety of cells including T cells, B cells, fibroblasts, endothelial cells, monocytes, keratinocytes, mesangial cells, and some tumor cells. The genes for human and murine IL-6 have been cloned and sequenced. Human IL-6 has a molecular mass of 21 to 28 kDa and is comprised of 212 amino acids that include two possible N-glycosylation sites and four cysteine residues.

IL-6 is a pleiotropic cytokine with multiple roles in the regulation of inflammation and hematopoiesis. IL-6 is produced at the site of inflammation and plays a key role in the acute phase response as defined by a variety of clinical and biological features such as the production of acute phase proteins.

IL-6 is the major regulator of the acute phase response in human hepatocytes. Due to its pleiotropic action, IL-6 has been intensively studied in many laboratories. It turned out to be an important factor in the immune and in the hematopoietic system and the major mediator in the hepatic acute phase response.

IL-6 is one of the proinflammatory cytokines and is detected in serum in the early stages of infections. Particularly in bacterial infections, IL-6 levels may be higher than CRP in early disease stages, and this may be helpful for early diagnosis. Early in infection, the CRP level may be low, but serial measurements can provide useful results and can be helpful in deciding when to discontinue antibiotic treatment. The combination of IL-6 and CRP has recently been proven to be useful in the early diagnosis of sepsis in newborns.

**PRINCIPLE**

This 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 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 instrument for ichroma tests to show IL-6 concentration in the sample.
ichroma™ II-6 will provide accurate and reliable results subject to the below conditions.
- ichroma™ II-6 should be used only in conjunction with instrument for ichroma™ tests.
- Have to use recommended anticoagulant sample.

Recommended anticoagulant
K₂EDTA, K₃EDTA, Lithium heparin

Storage condition

<table>
<thead>
<tr>
<th>Component</th>
<th>Storage Temperature</th>
<th>Shelf life</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cartridge</td>
<td>2 - 30 °C</td>
<td>20 months</td>
<td>Disposable</td>
</tr>
<tr>
<td>Detector tube</td>
<td>2 - 30 °C</td>
<td>20 months</td>
<td>Disposable</td>
</tr>
<tr>
<td>Detector diluent</td>
<td>2 - 30 °C</td>
<td>20 months</td>
<td>Opened</td>
</tr>
</tbody>
</table>

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 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 result as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or 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.

MATERIALS SUPPLIED

CFPC-116

Components of ichroma™ II-6

- Cartridge Box
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 1
  - 35 µL Capillary tube 25
  - ID chip 1
  - Instruction for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from ichroma™ II-6.
Please contact our sales division for more information.

- ichroma™ II
  REF FPRR021
- ichroma™ 50
  REF FPRR022
- ichroma™ III
  REF FPRR037
- Boditech II-6 Control
  REF CFPO-296

SAMPLE COLLECTION AND PROCESSING

The sample type for ichroma™ II-6 is human whole blood/serum/plasma.
- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- Samples may be stored for a week at room temperature or 2-8 °C prior to being tested.
- If testing will be delayed more than a week, samples should be frozen at -20°C ~ -70°C. Samples stored frozen at -20°C ~ -70°C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- Once the sample was frozen, it should be thawed one time and only for test, because repeated freezing and thawing can result in the changed test values.

[35 µL Capillary tube]
Fingertip blood sample should be collected below:

① Wear disposable gloves and the protective equipment for safety.
② Open the zipper bag which has capillary tubes.
③ Take out the 35 µL capillary tube and check for damaged or contamination.
④ Hold the handle of the 35 µL capillary tube and touch the surface of blood with the capillary tube.
⑤ Fill it with blood completely.
(Do not make air bubbles in the capillary tube and careful not to get blood on the surface of the capillary tube. If blood gets on the surface of the capillary tube, remove it gently with gauze.)

TEST SETUP

- Check the contents of ichroma™ II-6: Sealed Cartridges, Detector tubes, Detector diluent, Capillary tubes, ID chip and Instruction for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, detector diluent as well as an ID chip.
- If the sealed cartridge and the detection buffer 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.
(Please refer to the ‘Instrument for ichroma™ tests Operation Manual’ for the complete information and operating instructions).
**TEST PROCEDURE**

### ichroma™ II

**<Multi mode>**

1. Transfer 150 µL of the detector diluent using a pipette to a detector tube containing granules. When the granule form is completely dissolved in the tube, it becomes detection buffer.
2. Transfer sample 35 µL (human whole blood/serum/plasma/control) using a pipette to a detector tube. ※ If you use a capillary tube (35 µL), put it into the detector tube after collecting sample.
3. Close the lid of the detector tube and mix the sample thoroughly by shaking it about 20 times.
4. Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
5. Leave the Cartridge at room temperature for 12 minutes before inserting the device into the holder.

   * 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. Tap the ‘START’ button on the instrument for ichroma™ tests to start the scanning process.
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.

   * (Please refer to the ‘Instrument for AFIAS-50 tests Operation Manual’ for the complete information and operating instructions).

### <Single mode>

1. Transfer 150 µL of the detector diluent using a pipette to a detector tube containing granules. When the granule form is completely dissolved in the tube, it becomes detection buffer.
2. Transfer sample 35 µL (human whole blood/serum/plasma/control) using a pipette to a detector tube. ※ If you use a capillary tube (35 µL), put it into the detector tube after collecting sample.
3. Close the lid of the detector tube and mix the sample thoroughly by shaking it about 20 times.
4. Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
5. Insert the 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.
6. Tap the ‘START’ button on the instrument for ichroma™ tests.
7. Cartridge goes inside the Instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 12 minutes.
8. Read the test result on the display screen of the instrument for ichroma™ tests.

**ichroma™-50**

1. Insert the tip array in the tip station.
2. Insert the detector tube in the reagent station and cover the reagent station.
3. Open the lid of the detector diluent and insert the detector diluent in the diluent station.
4. Open the cover of the magazine station and pull and lift the cartridge magazine.
5. Insert the cartridges in the cartridge magazine individually.
6. Insert the cartridge-loaded cartridge magazine into the magazine station and close the cover of the magazine station.
7. Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
8. Tap the button located in the upper side of the No. of test cartridge region to select ID chip what you want to use.
9. When the selected cartridge slot is activated, set the number of test cartridge by tapping.
10. Tap the button located in the upper side of the No. of reagent region to select ID chip what you want to use.
11. When the selected slot is activated, set the number of detector tube by tapping.
12. Set the number of pipette tips by tapping.
13. Tap the ‘START’ button on the upper left of the main screen to start test.

   * (Please refer to the ‘Instrument for AFIAS-50 tests Operation Manual’ for the complete information and operating instructions).

### ichroma™ III

1. The test procedure is same with “ichroma™ II Single test mode ① – ⑧”.
2. Insert the sample-loaded cartridge into the ichroma™ III.
3. Tap the “Start” button on the ichroma™ III.
4. Cartridge goes inside and the ichroma™ III will automatically start scanning the sample-loaded cartridge after 12 minutes.
5. Read the test result on the display screen of the ichroma™ III.

**INTERPRETATION OF TEST RESULT**

- The instrument for ichroma™ tests calculates the test result automatically and displays IL-6 concentration of the test sample in terms of pg/mL.
- Reference value: 7 pg/mL
- Working range: 2 - 2,500 pg/mL

**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.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with ichroma™ IL-6. For more information regarding obtaining the control
materials, contact Boditech Med Inc.’s Sales Division for assistance.

( Please refer to the instruction for use of control material.)

## PERFORMANCE CHARACTERISTICS

### Analytical sensitivity

- LOB (Limit of Blank) 0.5 pg/mL
- LOD (Limit of Detection) 1.0 pg/mL
- LOQ (Limit of Quantitation) 2.0 pg/mL

### 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™ IL-6* test results did not show any significant cross-reactivity with these biomolecules.

<table>
<thead>
<tr>
<th>No.</th>
<th>Cross reactivity materials</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Interleukin-1α</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>2</td>
<td>Interleukin-1β</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>3</td>
<td>Interleukin-2</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>4</td>
<td>Interleukin-3</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>5</td>
<td>Interleukin-4</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>6</td>
<td>Interleukin-8</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>7</td>
<td>Interferon-γ</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>8</td>
<td>TNF-α</td>
<td>50 ng/mL</td>
</tr>
</tbody>
</table>

- **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™ IL-6* test results did not show any significant interference with these materials.

<table>
<thead>
<tr>
<th>No.</th>
<th>Interference materials</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bilirubin</td>
<td>342 µmol/L</td>
</tr>
<tr>
<td>2</td>
<td>Cholesterol</td>
<td>13 mmol/L</td>
</tr>
<tr>
<td>3</td>
<td>D-Glucose</td>
<td>55 mmol/L</td>
</tr>
<tr>
<td>4</td>
<td>Hemoglobin</td>
<td>2 g/L</td>
</tr>
<tr>
<td>5</td>
<td>L-Ascorbic acid</td>
<td>170 µmol/L</td>
</tr>
<tr>
<td>6</td>
<td>Triglyceride</td>
<td>37 mmol/L</td>
</tr>
<tr>
<td>7</td>
<td>EDTA</td>
<td>3.4 µmol/L</td>
</tr>
<tr>
<td>8</td>
<td>Heparin</td>
<td>3,000 U/L</td>
</tr>
</tbody>
</table>

### Precision

3 Lots of *ichroma™ IL-6* were tested for 21 days (7 days per 1 Lot at 1 site by one operator). Each standard material was tested 2 times per day. For each test, each material was duplicated.

- **Repeatability** (within-run precision)

  Repeatability of *ichroma™ IL-6* was evaluated with results of 1 Lot.

- **Total precision** (within-laboratory precision)

  Total precision (within-run, between-run, between-day) of *ichroma™ IL-6* was evaluated with results of 1 Lot.

- **Lot to lot precision**

  Lot to lot precision of *ichroma™ IL-6* was evaluated with results of 3 Lot.

<table>
<thead>
<tr>
<th>Conc. [pg/mL]</th>
<th>Repeatability</th>
<th>Total precision</th>
<th>Lot to lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVG</td>
<td>CV (%)</td>
<td>AVG</td>
<td>CV (%)</td>
</tr>
<tr>
<td>AVG</td>
<td>CV (%)</td>
<td>AVG</td>
<td>CV (%)</td>
</tr>
<tr>
<td>AVG</td>
<td>CV (%)</td>
<td>AVG</td>
<td>CV (%)</td>
</tr>
<tr>
<td>AVG</td>
<td>CV (%)</td>
<td>AVG</td>
<td>CV (%)</td>
</tr>
<tr>
<td>9</td>
<td>9.03</td>
<td>5.8</td>
<td>9.07</td>
</tr>
<tr>
<td>42.61</td>
<td>42.35</td>
<td>6.7</td>
<td>42.43</td>
</tr>
<tr>
<td>1274</td>
<td>1270.3</td>
<td>6.4</td>
<td>1265.2</td>
</tr>
</tbody>
</table>

### Accuracy

The accuracy was confirmed by testing with 3 different lots of *ichroma™ IL-6*. The tests are repeated 10 times in each different concentration.

<table>
<thead>
<tr>
<th>Conc. [pg/mL]</th>
<th>LOT 1 AVG</th>
<th>LOT 2 AVG</th>
<th>LOT 3 AVG</th>
<th>AVG</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>8.34</td>
<td>8.8</td>
<td>9.06</td>
<td>8.73</td>
<td>-3.00%</td>
</tr>
<tr>
<td>42.61</td>
<td>42.77</td>
<td>41.74</td>
<td>41.45</td>
<td>41.99</td>
<td>-1.50%</td>
</tr>
<tr>
<td>1274</td>
<td>1203.8</td>
<td>1244.1</td>
<td>1300.5</td>
<td>1249.5</td>
<td>-1.90%</td>
</tr>
</tbody>
</table>

### Comparability

IL-6 concentrations of 118 samples were quantified independently with *ichroma™ IL-6* (*ichroma™ II*) and Comparator A as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were

\[
y = 0.9355x + 6.6376 \\
R= 0.9967
\]

7. Interleukin-6: A sensitive parameter for the early


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>⬤</td>
<td>Sufficient for &lt;n&gt; tests</td>
</tr>
<tr>
<td>📖</td>
<td>Read instruction for use</td>
</tr>
<tr>
<td>🕒</td>
<td>Use by Date</td>
</tr>
<tr>
<td>📄</td>
<td>Batch code</td>
</tr>
<tr>
<td>💰</td>
<td>Catalog number</td>
</tr>
<tr>
<td>⚠️</td>
<td>Caution</td>
</tr>
<tr>
<td>🌐</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>🔐</td>
<td>Authorized representative of the European Community</td>
</tr>
<tr>
<td>⚫️</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>℃</td>
<td>Temperature limit</td>
</tr>
<tr>
<td>🔴</td>
<td>Do not reuse</td>
</tr>
</tbody>
</table>

For technical assistance; please contact:
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E-mail: sales@boditech.co.kr

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