ichroma™ IL-6

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 the 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.

COMPONENTS

ichroma™ IL-6 consists of 'Cartridges', 'Detector tubes', 'Detector diluent', 'Capillary tubes', 'ID chip', and 'Instruction for use'.

- The cartridge contains the membrane called a test strip which has streptavidin at the test line, and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The detector tube has 2 granules containing anti-chicken IgY fluorescence conjugate, anti-Interleukin 6 fluorescence conjugate, anti-Interleukin 6 biotin conjugate, mouse IgG, bovine serum albumin(BSA), bromophenol blue and sucrose as a stabilizer and sodium azide as a preservative in Tris buffer and phosphate buffered saline. All detector tubes are packed in a pouch.
- The detector diluent contains bovine serum albumin(BSA), sodium chloride, Tween20, CA-630, sodium azide as a preservative in phosphate buffered saline. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent, capillary tube, and ID chip) must match each other.
- Keep the lid closed of detector diluent after using detector diluent.
- 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 cartridge or detector tube. 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 cartridge, if pouch is damaged or has already 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.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent, capillary tubes and pipette tips should be handled carefully and discarded by an appropriate measure 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.
- **ichroma™ IL-6** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ IL-6** 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, Lithium heparin

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	4 - 30 °C	20 months	Disposable
Detector tube	4 - 30 °C	20 months	Disposable
Detector diluent	4 - 30 °C	20 months	Unopened
	4 - 30 °C	20 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 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

REF CFPC-116

Components of **ichroma™ IL-6**

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

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ IL-6.**

Please contact our sales division for more information.

- **ichroma™ II** REF FPRR021
- **ichroma™50** REF FPRR022

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ IL-6** is human whole blood /serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- Take precautions on the collected sample because it is reported the concentration is rapidly changed when the sample for IL-6 test is kept at room temperature or refrigerated.
- If longer storage is required, e.g. if the test could not be performed within 24 hours, serum or plasma should be immediately frozen below -20 °C. The freezing storage of sample up to 3 months does not affect the quality of results.
- Do not freeze whole blood sample 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.

TEST SETUP

- Check the contents of **ichroma™ IL-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.

<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 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 left upper of the main screen to start test.

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

- The Quality control tests should be used to confirm the reliability and the validity of **ichroma™ IL-6**.
- 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 be performed both to verify proper operation of instrument and to exclude any possible performance change in storage.
- For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.

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.

No.	Cross reactivity materials	Conc.
1	Interleukin-1α	50 ng/mL
2	Interleukin-1β	50 ng/mL
3	Interleukin-2	50 ng/mL
4	Interleukin-3	50 ng/mL
5	Interleukin-4	50 ng/mL
6	Interleukin-8	50 ng/mL
7	Interferon-γ	50 ng/mL
8	TNF-α	50 ng/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™ IL-6** test results did not show any significant interference with these materials.

No.	Interference materials	Conc.
1	Bilirubin	342 umol/L
2	Cholesterol	13 mmol/L
3	D-Glucose	55 mmol/L
4	Hemoglobin	2 g/L
5	L-Ascorbic acid	170 umol/L
6	Triglyceride	37 mmol/L
7	EDTA	3.4 umol/L
8	Heparin	3,000 U/L

- **Precision**

  3 Lots of **ichroma™ IL-6** were tested for 21days (7days 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)
    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.

Conc.	Repeatability		Total precision		Lot to lot	
[pg/mL]	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
9	8.94	6.6	9.00	6.4	9.04	6.4
42.61	42.21	6.6	42.11	6.2	42.15	6.3
1274	1295.1	6.5	1294.4	6.3	1279.6	6.0

  - Between person
    Three different persons tested **ichroma™ IL-6** , ten times at each concentration of the control standard.
  - Between site
    One person tested **ichroma™ IL-6** at three different sites, ten times at each concentration of the control standard.

Conc.	Between-person		Between-site	
[pg/mL]	AVG	CV (%)	AVG	CV (%)
9	8.82	7.3	8.92	6.8
42.61	41.42	7.2	43.07	6.3
1274	1233.99	7.5	1244.83	7.2

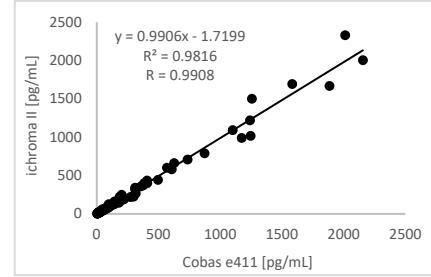
- **Accuracy**

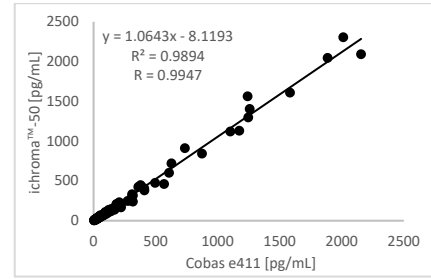
Conc. [pg/mL]	LOT 1	LOT 2	LOT 3	AVG	Bias (%)
9	8.34	8.8	9.06	8.73	-3.00%
42.61	42.77	41.74	41.45	41.99	-1.50%
1274	1203.8	1244.1	1300.5	1249.5	-1.90%

- **Comparability**

  IL-6 concentrations of 100 clinical were quantified independently with **ichroma™ IL-6** and Cobas e411 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 are the below.

	Cobas e411	
	Linear regression	Coefficient of correlation (R)
ichroma™ II	y = 0.9906x - 1.7199	0.9908
ichroma™-50	y = 1.0643x - 8.1193	0.9947





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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

For technical assistance; please contact:
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