ichroma™ Total IgE

INTENDED USE

ichroma™ Total IgE is a fluorescence immunoassay (FIA) for the quantitative determination of total IgE (Immunoglobulin E) in human whole blood/serum/plasma. It is useful as an aid in diagnosis and management of allergic disease.

For *in vitro* diagnostic use only.

INTRODUCTION

Immunoglobulin E (IgE) was discovered for its involvement in allergic reactions (Type I hypersensitivity)[1]. Type I hypersensitivity is an allergic reaction provoked by re-exposure to a specific type of antigen referred to as an allergen.

The sequence of events in the allergic reaction consists of the production of IgE antibodies in response to an allergen, binding of IgE to of mast cells, cross-linking of the bound IgE by the allergen upon re-exposure, and release of mast cell mediators such as histamine, lipid mediators and cytokines. Some mast cell mediators cause rapid increase in vascular permeability and smooth muscle contraction, resulting in many of the symptoms.

The IgE concentration in serum is normally very low (<0.001% of the total serum immunoglobulin). The serum concentration of IgE is age- related, increasing during childhood until about 10 years of age, after which it reaches values that are maintained during adult life [2), 3)].

Measurement of total IgE is often used as a tool in the diagnosis and management of atopic diseases, and elevated level of IgE can be found in patients with allergic disease such as asthma, hay fever, atopic dermatitis and urticarial [4), 5), 6)].

It has been used to distinguish atopic from non-atopic individuals presenting allergy-like symptoms. In addition, studies have also shown that increased levels of IgE in cord blood and infants may be predictive of future atopic tendencies [7)].

Serum IgE levels may vary as a result of diet, genetic background, geographical location and other factors. It is therefore recommended that total IgE measurements be used in conjunction with other clinical tests when establishing diagnoses [8)].

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-streptavidin 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 total IgE concentration in the sample.

COMPONENTS

ichroma™ Total IgE consists of 'cartridges', 'detector tube' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has anti- IgE at the test line, and streptavidin 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-IgE conjugate and sodium azide as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in a pouch.
- The detector diluent contains sodium azide as a preservative in phosphate buffer saline (PBS), and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the 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 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 it may cause certain health issues like convulsions, low blood pressure, low 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™ Total IgE** when biotin concentration in the sample was below 10 ng/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™ Total IgE** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Total IgE** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Na_2 EDTA, K_2 EDTA, K_3 EDTA, Lithium heparin, Sodium citrate

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2-30°C	20 months	Disposable
Detector diluent	2-30°C	20 months	Disposable
Detector diluent	2-30°C	20 months	Unopened
		3 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 antigens 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 antigens with time and/or temperature may also cause false negative result as it makes the antigens unrecognizable by the antibodies.
- An interference can be found for samples from patients treated with Xolair (omalizumab) or similar drugs containing anti IgE 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 in conjunction with clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-91

Components of **ichroma™ Total IgE**

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

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Total IgE**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M3** REF FPRR035
- **Boditech Total IgE Control** REF CFPO-219

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Total IgE** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) may be stored for a month at 2-8 °C prior to being tested. If testing will be delayed more than a month, 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.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ Total IgE**: 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 tube, the detector diluent as well as an ID chip.
- If the sealed cartridge, the detector tube and the 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™ II, ichroma™ M3**

Multi test mode

1) Take 150 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 50 µL of sample (serum/plasma/control) or 100 µL of sample (whole blood) 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 12 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) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M3 will start the test 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) Tap the 'Start' button on the instrument for ichroma™ tests.
(ichroma™ M3 will start the test automatically after inserting.)
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 12 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 total IgE concentration of the test sample in terms of IU/mL.
- To convert the IU/mL of the result to mass unit per volume, the conversion factor can be used as follow:
1 IU/mL = 2.44 ng/mL
- The concentration of IgE in the serum is highly age dependent.
- Total IgE concentrations were measured in human serum samples from non-atopic healthy adult and child subjects using the **ichroma™ Total IgE**. The observed ranges of total IgE concentrations are shown below for each age group represented:

Age group	Geometric mean *	ichroma™ Total IgE
		Mean + 1SD
< 1 year	3.2 IU/mL	13.6 IU/mL
1 – 5 years	12.1 IU/mL	43.3 IU/mL
6 – 9 years	20.6 IU/mL	80.1 IU/mL
10 – 15 years	51.1 IU/mL	209.2 IU/mL
≥ 16 years	13.2 IU/mL	88.4 IU/mL

* Reference values for serum IgE in healthy non-atopic children and adults. *Clin Chem.* 1982;**28**(7):1556.

- Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference ranges.
- Working range: 1.00 IU/mL – 1,000 IU/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.
- 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™ Total IgE**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
(Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 0.50 IU/mL
  - Limit of Detection (LoD) 0.75 IU/mL
  - Limit of Quantitation (LoQ) 1.00 IU/mL
  - Reportable range
  Reportable range of the undiluted sample is 1.00 IU/mL – 1,000 IU/mL. Samples with total IgE concentrations above 1,000 IU /mL can be diluted with saline (0.9% NaCl in distilled water, not provided). The recommended dilution is 1:10 or 1:100.
  After dilution, multiply the result by the dilution factor. Please follow the below equation to obtain final sample concentration.
  [Final sample conc. = Reported conc. x Dilution factor (10 or 100)]

- **High-dose Hook Effect**
There is no high dose hook effect at IgE concentration up to 15,000 IU/mL.

- **Analytical specificity**
  - Cross-reactivity
  Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ Total IgE** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactants	Concentration
Human IgG	20 mg/mL
Human IgM	20 mg/mL
Human IgA	20 mg/mL

- Interference
  Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ Total IgE** test results did not show any significant interference with these materials.

Interferents	Concentrations
Hemoglobin	200 mg/dL
Bilirubin	0.4 mg/mL
Triglyceride	2,000 mg/dL
Rheumatoid factor	78 IU/mL
Human serum albumin	12 g/dL

■ **Precision**

- **Single-site study**
  Repeatability (within-run precision)
  within-laboratory precision (Total precision)
  Lot to lot precision
  3 Lots of **ichroma™ Total IgE** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study				
Total IgE [IU/mL]	Repeatability		Within-laboratory precision	
	AVG [IU/mL]	CV (%)	AVG [IU/mL]	CV (%)
5.00	5.13	7.50	5.07	7.55
100.00	98.65	8.04	100.71	7.89
500.00	505.61	6.58	496.84	7.39

Single-site study		
Total IgE [IU/mL]	Lot to lot precision	
	AVG [IU/mL]	CV (%)
5.00	5.07	8.05
100.00	100.13	7.78
500.00	502.01	7.42

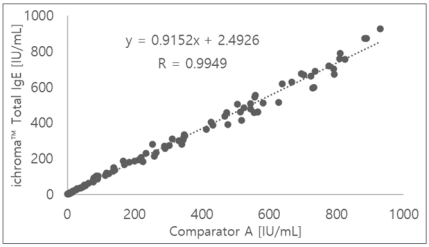
- **Between persons**
  3 Lots of **ichroma™ Total IgE** were tested 10 times by 3 different persons.
- **Between sites**
  1 Lot of **ichroma™ Total IgE** was tested 10 times at 3 different sites.

Total IgE [IU/mL]	Between persons		Between sites	
	AVG [IU/mL]	CV (%)	AVG [IU/mL]	CV (%)
5.00	5.01	7.89	5.20	8.31
100.00	98.76	8.62	98.35	7.70
500.00	502.56	7.48	501.18	8.71

■ **Accuracy**
The accuracy was confirmed by testing with 3 different lots of **ichroma™ Total IgE**. The tests were repeated 10 times at each concentration of the control standard.

Total IgE [IU/mL]	Lot 1	Lot 2	Lot 3	AVG [IU/mL]	Recovery (%)
5	4.90	4.85	5.25	5.00	100%
12.5	12.11	12.77	12.08	12.32	99%
25	25.68	24.27	24.71	24.89	100%
50	51.44	49.43	51.93	50.93	102%
100	100.4	94.7	104.2	99.78	100%
250	250.5	246.4	253.0	250.0	100%
500	496.1	516.5	496.5	503.0	101%
600	595.0	590.6	591.7	592.4	99%
700	711.5	698.2	674.2	694.6	99%
800	772.6	783.0	807.4	787.7	98%
900	882.6	893.1	913.5	896.4	100%

■ **Comparability**
Total IgE concentration of 100 clinical samples were quantified independently with **ichroma™ Total IgE (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 follow.



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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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